Annals
of the
Missouri Botanical Garden

VOL. 5 NOVEMBER, 1918 No. 4

ETHERIZATION OF TISSUES AND ITS EFFECT ON ENZYME ACTIVITY

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In the years following the discovery of anaesthesia by ether and other substances a great amount of work has been devoted to the study of the stimulating and inhibiting effects of various factors on plants and animals; the literature on the subject is voluminous. A knowledge of the range of chemical compounds capable of producing a form of either stimulation or anaesthesia (and we shall see that these contrasting phenomena are closely allied with respect to the causal agent) has vastly increased, and embraces many substances, both organic and inorganic, which do not ordinarily come to mind as anaesthetics. No attempt will here be made to review, except incidentally, the work in the domain of animal physiology bearing on the anaesthesia question; neither is it germane to the present study to consider more fully the literature relating to stimulation and inhibition caused by other agents than those commonly regarded as anaesthetics, i. e., ether, chloroform, and chemically related substances.

SURVEY OF LITERATURE

EFFECT ON IRRITABILITY

Effect on growth and turgor movements of complex members.—Probably the first experiments with ether in the field of plant physiology are those of Clemens ('47, '48, '48[a]) and of Marcet.[1] The former found that *Mimosa pudica* and

[1] Cited by Hempel ('11) and by Rothert ('03).

stamens of *Berberis* lost their sense of irritability in vapor of acetic or sulphuric ether. Leclerc ('53), also studying *Mimosa,* noted a like loss of irritability, with subsequent recovery when the dose was not excessive, and concluded that plants possessed a nervous system analogous to that of animals. Loss of irritability varied with light conditions, being accelerated in direct sunlight. Kabsch ('61) noted that the periodic movement of the leaflets of *Hedysarum gyrans* L. was inhibited by ether and by chloroform and that these vapors were lethal in their effect except in minimal doses of the former. *Mimosa pudica* was still a favorite object of study, indicated by the work of Blondeau ('67), who confirmed Leclerc and came to similar conclusions, and by the observations of Bert ('67), who concluded that the paratonic movements alone were affected by etherization. Pfeffer ('73) did not subscribe to this view, since he found that sleep movements also were suspended by such treatment.

Carlet ('73) observed a retardation in nutation of the anthers of *Ruta* when subjected to ether or chloroform, although movement was not completely inhibited. Under the influence of the anaesthetics the pollen sacs did not open. Heckel ('73) found that stamens of *Berberis* lost the power of irritability in a chloroform atmosphere, while those of *Ruta* were unaffected, and he supported Bert's view of two classes of plant movements—spontaneous and induced. Heckel's observations along this line ('74, '74[a], '74[b], '74[c], '74[d], '74[e]) led him to conclusions regarding the seat of response to anaesthetics which Pfeffer ('75) has criticized as based on errors of technique and misinterpretation of factors involved. A later study by Heckel ('76) of *Drosera* and *Pinguicula* was concerned with variations produced by different doses of the anaesthetics. Darwin ('75) studied the effect of ether and chloroform upon the movement of the tentacles of *Drosera,* noting loss of irritability and subsequent recovery when restored to normal conditions. The classic experiments of Bernard ('78) also included the response of *Mimosa* to anaesthetics.

Siragusa ('79), noting the effect of ether on various manifestations of higher and lower plants, saw that the spontaneous periodic opening and closing of flowers was inhibited in an atmosphere of that vapor. Arloing ('79) substantiated the previous findings with respect to *Mimosa*. Macchiati ('80) concluded that the inhibitory action of chloroform and ether upon stamen movements in *Ruta* and *Smyrnium* was due to the temperature reduction effected by the vapors, although Cugini ('81) attempted to disprove this point by experiments with the same anaesthetics under super-normal temperatures, to which Macchiati ('83) took exception. Tassi ('84, '87) followed with a study of the effect of volatile and non-volatile anaesthetics on cut flowers in conditions of diffuse light. He observed a paralyzing effect upon perianth movements. Temperature reduction apparently played no rôle in explaining the results, since similar action occurred with the non-volatile substances. Macchiati ('84), however, endeavored to show that these results were open to question because of the use of anaesthetic dosages of lethal concentration.

References to the effect of anaesthetics on special tropic response are not numerous. Molisch ('84) reported that the roots of maize seedlings were negatively tropic to atmospheres of nitrous oxide, chloroform, ether, and other vapors and gases. Czapek ('98) in his extensive study of geotropic stimuli found that chloroform in aqueous solution increased the geotropic induction time as well as the reaction time for roots of *Vicia* and *Lupinus* seedlings. Bertel ('02) noted a geotropic after-effect of chloroform on the roots of *Lupinus* seedlings in the course of metabolism studies. Wächter ('05) reported what he designated as chemonastic response in the case of *Callisia repens* where the leaves were made to droop notably as the result of exposure to ether, illuminating gas, and other gaseous atmospheres.

Effect on microörganisms. — The field of microbiology, using this term in a very general sense, has received some attention with respect to the effect of stimulants and anaesthetics on functional and tactic responses. Thus Bernard ('78) recorded the inhibition of dextrose fermentation by

yeast in presence of chloroform. Siragusa ('79) reported the failure of long ether exposure on subsequent activities of bacteria, yeasts, and molds, but this early work may be questioned. Elfving ('86), studying yeasts among other objects, noted increased carbon dioxide production by such organisms from dextrose and asparagin in presence of narcotics. Swarm spores of *Chlamydomonas pulvisculus* showed a reversal of phototactic response from negative to positive in the presence of ether. Chloroform, on the other hand, proved toxic.

Rothert ('03) essayed to determine tactic effects of anaesthetics on microörganisms as distinct from effect on motility, and reported varying chemotactic inhibition effects of ether. *Bacillus Solmsii* had its chemotactic response inhibited by this substance, but not by chloroform. *Gonium pectorale,* on the other hand, was partially affected as to phototaxy by chloroform. Rothert also recorded a noteworthy reversal of phototactic response (negative to positive) and a shifting of the light intensity optimum. An osmotactic susceptibility was completely inhibited by a narcosis which only inhibited chemotaxy and "aërotaxy" to varying degrees. While motility was found to depend on length of exposure, anaesthetic effects were held to bear a relation solely to the concentration used. A distinction is thus made between a narcotic and a progressive toxic action.

The results of the studies of Herzog and Hörth ('07) on the effects of vapors on yeast autolysis will be considered under another heading. The fermentation experiments of Koch ('11) indicated a definite stimulus to activity of yeasts by small ether dosages. Fred ('11) also noted a stimulus to bacterial growth effected by ether in small concentrations, and even reported an increased nitrogen-fixing capacity of *Azotobacter* in pure culture as a result of etherization. Harvey ('15) has found that when ether or chloroform, among a series of compounds tested, was added to tubes containing a sea-water emulsion of luminous bacteria, the light emitted by the organisms disappeared almost immediately. He does not state, however, whether the action is reversible, as was the case with various alcohols used.

Effect on protoplasmic streaming. — Ewart ('03) cites Kühne as probably the first to show that chloroform and ether inhibit protoplasmic streaming. Dilute solutions at first caused stimulation, while concentrations of 10–25 per cent saturation caused immediate retardation. The reversal of the action, if the normal environment was restored after a short inhibition period, was also substantiated by Hauptfleisch ('92). Retardation of streaming in hairs of *Primula,* *Petunia,* and *Lycopersicum* was also reported by Schneider ('93). Demoor ('95) noted the initial stimulus of chloroform on the protoplasmic movement of *Tradescantia,* with marked vacuolization for a short period preceding anaesthesia. Pre-anaesthesial excitation of the nucleus, he observed, was greater than that of the cytoplasm. Recovery of normal functions varied with the individual objects studied, which included cells of *Fumaria,* and leucocytes.

The observations of Farmer and Waller ('98) are in general a confirmation of the work already cited. The materials studied in this case were *Chara* and *Elodea.* On the other hand, the findings of Demoor are controverted in part by Samassa ('98–'01?), who noted that with complete inhibition of protoplasmic streaming in *Tradescantia,* nuclear division does not continue, but an inhibitory after-effect of varying duration results.

Josing ('01) approached the problem from another viewpoint, considering the effect of the anaesthetics, among other factors, with respect to the environment. *Vallisneria, Elodea,* and *Trianea* were studied, and treatment resulted in cessation of streaming in darkness and a renewal of activity in light. An aqueous solution of ether of definite concentration increased the length of time of streaming in light. Deviation from this optimum on either side resulted in time reduction. Streaming under unfavorable temperature conditions continued for a longer period in etherized plants than in controls. In the absence of oxygen or in the presence of carbon dioxide the period of streaming in control plants was greater than in those previously etherized.

EFFECT ON GROWTH ELONGATION AND CELL DIVISION

Growth responses to the agents with which we are here concerned have also occupied the attention of several botanists. Siragusa ('79) noted growth inhibition of both aërial and subterranean organs of higher plants. Detmer ('82) also found that chloroform inhibited the growth of *Pisum* and *Triticum* or impeded it notably, with reduced respiration. Elfving ('86) reported inhibition of growth of sporangiophores of *Phycomyces nitens* for short periods as the result of ether treatment, without subsequent recovery. Bateson and Darwin ('87) studied the effect of ether and chloroform vapors on pith elongation of *Helianthus,* using auxanometric methods, and found that ether effected a distinct increase in length, varying with the dosage employed. Chloroform had a variable effect, acting as a stimulant in one case; with weaker dosage, however, there was either inhibition or no effect.

Townsend ('97) subjected leaves of *Avena sativa* to ether atmospheres of different concentrations for varying periods. Retardation of growth was found to vary with increase of ether concentrations in the atmosphere. Weak concentrations first produced a retardation, followed by a distinct stimulus. In some cases the stimulus to growth did not become evident for a day, and then lasted throughout the period of the experiment. Longer exposure resulted in retardation for two days, followed by a return to normal growth rate without any intermediate period of acceleration. Sandsten ('98) also recorded a growth acceleration caused by weak ether and chloroform dosages on seedlings of *Zea Mays,* and a retarding action of stronger concentrations. Resting bulbs were killed by long exposure to weak doses. Latham ('05) studied the relation of chloroform to the growth of *Sterigmatocystis nigra* and of *Penicillium glaucum.* She found the usual concentration relations to hold,—growth stimulation with small doses and inhibition or death following the use of large ones. The effect of a given dose increased with rise in temperature. The increased growth observed was attributed to increased

metabolic economy, on the basis of less sugar consumed and less acid formed.

Burgerstein ('06) found that a very low ether content in air (.004 per cent) accelerated the hypocotyl growth of *Phaseolus, Cucurbita,* and *Helianthus,* while the same chloroform dose produced varying degrees of inhibition. Finally, Schroeder ('08) studied the effect of ether on the growth of *Avena sativa* seedlings, using the horizontal microscope under constant temperature conditions. He concluded that the effect of ether upon growth was a function of concentration and length of exposure. The first effect was stimulation, followed by a subnormal depression. With increased dosage the latter was more marked and occurred more rapidly until concentrations were reached where growth inhibition was immediate and death resulted.

Studies of anaesthesial action on cell structure have been reported by Nathansohn ('00), Sabline ('03), von Wasielewski ('04), and Gerassimow ('05). Nathansohn studied the effect of ether solutions on *Spirogyra* and *Closterium* and observed abnormal nuclear divisions in the latter. *Spirogyra* showed neither form of mitosis under the influence of ether. Sabline concluded from a study of various chemical agents employed that sulphuric ether effected abnormal cytologic changes in the nuclear stages of root cells of *Vicia Faba.* Von Wasielewski obtained amitotic figures in root tips of the same plant with 0.5 and 1 per cent chloroform water, but negative results with 1 per cent ether solutions, although in the latter case he observed an increase in the number of normal mitoses. Gerassimow believed from his study of *Spirogyra* in ether cultures that the increase in thickness of the cell, which occurred only where nuclei were present, indicated nuclear stimulation. Weak ether dosages increased response to stimuli and accelerated budding and general metabolic processes.

EFFECT ON GERMINATION

Observations on germination with respect to anaesthesia treatment occur in the literature, beginning with the work of Claude Bernard ('78), who noted the inhibitory action of

ether on sprouting seeds. Siragusa ('79) confirms such inhibition, but also points out the subsequent recovery therefrom. Giglioli ('82) studied the action of gases and liquids on the vitality of seeds, chloroform and ether being among a large number of substances tried. *Medicago sativa* showed a high resistance to both the anaesthetics in liquid form according to the time of treatment,—showing germination of 29 per cent after 484 days' immersion. Romanes ('94) found no appreciable effect on the germination of various seeds subjected to ether or other vapors for long periods, the seeds having been previously stored *in vacuo* for several months.

Townsend ('99) showed that while weak doses of ether accelerated the germination of cereals, vegetable seeds, and fungous spores, stronger ones either retarded or completely inhibited such action. Recovery of activity was also noted when normal environment was restored. Coupin ('99) compared the resistance of dry seeds to inhibitory action of saturated atmospheres of ether and chloroform with that of seeds previously subjected to moist conditions. The former were unaffected; the latter showed inhibition or death from treatments as weak as 37/10000. Duggar ('01) found chloroform to be lethal in one-half hour to spores of *Aspergillus* and *Phycomyces*. Ether had little effect as a stimulus, except in low concentrations, on *Aspergillus*. Opposed to Coupin were the findings of Schmid ('01), who reported that chloroform vapor was lethal to the protoplasm of latent seeds; that the seed-coats in that state were permeable to the anaesthetic vapor to varying degrees, and that injury varied with such permeability. Dixon ('02), in checking the work of Giglioli on the resistance of seeds to toxic agents, found that the resistance to chloroform and other poisons, as indicated by germination after treatment, depended on the integrity of the seed-coat.

Becquerel ('05), in the face of such contradictory findings, attacked the question anew, using seeds of wheat, lucerne, clover, peas, and lupine, both air-dried and dried to constant weight, with seed-coats injured and intact. His results also tended to show that lack of injury depends largely upon the

integrity of the seed-coat. Seeds with uninjured coats remained viable after subjection to anaesthetic vapors or solutions for almost a year; those with perforated coats were killed. Burgerstein ('06) also opposed Schmid's view by reporting that 24 hours' exposure of seeds of *Phaseolus, Cucurbita, Helianthus, Zea,* etc., to ether vapor resulted in germination stimulus, while seeds with previously imbibed water merely suffered a retardation. Chloroform in the same respective dosage was found to be more toxic. The germination of barley was stimulated, according to Kiessling ('11), by subjection to ether vapor for 80 minutes; a longer treatment resulted in a reduction in rapidity and per cent of germination. Hempel ('11), on the contrary, found that ether retarded the germination of *Pisum* seedlings.

EFFECT ON PERIODICITY

Under this heading may be grouped the investigations dealing with the forcing effect of anaesthetics on growth activity, as distinct from effects on germination as a phase of growth. Such forcing action of ether vapor in breaking or suspending the period of inactivity in the annual cycles of many plants has been the subject of considerable experimentation since Johannsen ('06) first called attention to the subject. A large part of the literature on the subject deals with methods and results as applied in the floricultural and horticultural field, and no review of the publications bearing on this phase is here attempted. Mention may be made of the work of Aymard ('04), Howard ('06, '10, '15, '15[a], '15[b], '15[c]),—who has devoted a considerable amount of time to experimental work along this line with twigs, potted plants, bulbs, herbaceous perennials, and seeds,—and the experiments of Stuart ('10).

Behrens ('06) regarded the shortening of the rest period of seeds by ether vapor as a pure stimulus effect independent of seed-coat permeability. Burgerstein ('06) noted a forcing action on bulbs of *Narcissus* with the same ether dosage that caused inhibition in *Allium Cepa.* Tulips, he reported to be unaffected by the anaesthetic. Jesenko ('11) varied the

experimental method by forcing solutions of ether under pressure into the cut ends of twigs. The lower concentrations used acted as stimulants, causing bud development if treatment occurred during the normal rest period of the plant; otherwise action was injurious.

EFFECT ON TRANSPIRATION

In the field of transpiration studies Jumelle ('90, '90[a]) found that the effect of ether in darkness was to decrease the action, while the same dosage had an accelerating influence in the light. He concluded that the anaesthetic acted on the chlorophyll and increased transpiration by converting all the energy in that direction, while assimilation was inhibited. His findings appear to be confirmed by Lommen in a brief note by MacMillan ('91), the former having measured the loss in weight of *Selaginella* following etherization. Schneider ('93) explained the increased water loss observed in his experiments as evaporation from tissues killed by the anaesthetic. Woods ('93), however, points out the error in these deductions, and on the basis of his investigations on *Canna indica* and *Mnium sp.* confirmed Jumelle's findings and explained the increased water loss in light as compared with plants in darkness purely on the physical basis of increased evaporation in daylight from the living tissues.

Darwin ('98) in an extensive study of stomatal response found that both chloroform and ether vapors caused a partial closing of stomata without subsequent injury. A careful study of anaesthetic effects with reference to water loss as related to plant activity was made by Dixon ('98), who gave special attention to the physical factors involved. Dixon found that ether and chloroform produced inhibition in both rate and amount of transpiration of cuttings, in contrast to an acceleration effected by oxygen. When, however, the specific transpiration in various atmospheres, based on air as standard, was compared with the specific evaporation of water in atmospheric currents of the same substances it appeared that, with the exception of oxygen, there were no marked differences within limits not lethal to the plants. The

conclusion was reached that anaesthetics among other vapors are without effect on photosynthetic action of leaf cells with respect to transpiration.

EFFECT ON RESPIRATION AND PHOTOSYNTHESIS

The study of the respiratory function as influenced by anaesthetic action has been a subject of inquiry since the days of Bernard. Gayon ('77) reported complete inhibition of respiration ("fermentation intracellulaire") of fruits by ether and chloroform. Schwarz ('81) reported that the presence of these substances in water effected a cessation of oxygen evolution and carbon dioxide assimilation in *Elodea* and *Ceratophyllum* without subsequent recovery. This opposed Bernard's conclusions ('78) that chloroform inhibited assimilation while respiration continued. Bonnier and Mangin ('86), using their method of gas analysis, were able to show that with the use of a measured amount of ether carefully added, carbon dioxide assimilation was checked without affecting respiration, thus confirming Bernard and opposing Schwarz.

Pringsheim ('87), in a study of assimilation and respiration of chlorophyllous plants, using the bacterium method, found that ether and chloroform inhibited assimilation, that such action was accompanied by death of the cells, and that the chlorophyll apparatus was changed.

Laurén ('91) studied the effect of ether on the respiration of various seedlings, and determined both aërobic and anaërobic respiration. Different plants gave varying response. The normal respiration of *Ricinus* and *Lupinus* was increased; of *Pisum, Phaseolus,* and *Cucumis* increased to a maximum with a certain per cent concentration, and decreased with higher ones. *Brassica, Hordeum,* and *Zea* were unaffected by weak dosages and inhibited by stronger ones. The anaërobic respiration of *Lupinus* and *Pisum* was increased, while this was not noted in *Ricinus, Zea,* and *Hordeum.* No explanation was offered for these differences, but attention was called to the fact that seedlings rich in carbohydrates

showed no respiratory response in comparison with those containing a high per cent of protein.

Ewart ('96), in his early work on this subject, came to conclusions similar to those of Schwarz. Kny ('97) found that *Spirogyra* anaesthetized with chloroform water until plasma movement had ceased, the nucleus become swollen, and the chloroplasts distorted, still retained its chlorophyllous functions. He concluded that injury to cytoplasm and nucleus was not directly correlated with the chlorophyll function. Ewart ('98), in a later study of *Elodea canadensis,* confirmed Bernard's findings of assimilatory inhibition without permanent injury. Téodoresco and Coupin ('98) studied the effect of ether on etiolated seedlings of wheat, vetch, lupine, and buckwheat. Chlorophyll formation was prevented or retarded according to conditions of dosage or length of exposure. Kauffmann ('99), studying the effect of narcotics on the protoplasmic processes with respect to chlorophyll formation, also showed that inhibition or permanent injury was dependent on strength of dosage and length of exposure. Morkowine ('99), from his experiments, opposed the findings of Bonnier and Mangin, whose error, he believed, resulted from insufficient periods of observation. Morkowine found that respiration intensity increased notably two hours after treatment. Zalenski ('02) also reported increased carbon dioxide evolution from corms of *Gladiolus* following an etherization of 1 cc. per 750 cc. volume, with a subsequent return to normal rate; stronger dosage caused depression. Exposure was shown to be a factor, since a dosage that stimulated after short exposure caused depression when the time of action was prolonged. Kosiński ('02) subjected *Aspergillus niger* to chemical and mechanical stimuli in absence of nutrients; ether dosages to a maximum of 2 per cent increased the respiration, higher ones depressed it, and the presence of 5 per cent in the nutrient solution caused immediate cessation of respiratory activity. Morkowine ('03) in a later paper reported experiments with *Vicia Faba, Beta vulgaris,* and *Gladiolus,* using various stimuli. Ether, among other compounds, showed a varying effect upon carbon dioxide evolution with

respect to intramolecular respiration. A minimum, optimum, and maximum stimulation were evident which were correlated with changes in the intensity of intramolecular respiration. It was found that under the stimulus plants could develop an anaërobic respiration equal to, or greater than, the normal respiration value. The ratio of anaërobic to normal respiration, however, was not found in general to change under the effect of stimuli.

Treboux ('03) concluded that ether and chloroform in weak concentrations increased both growth and respiration of *Elodea,* but did not have a similar effect on assimilation; a suitable dose of chloroform caused assimilation to be temporarily inhibited. Kegel ('05) found reduction or complete inhibition of carbon dioxide evolution in *Elodea canadensis* with chloroform of varying concentrations. Solutions of .4–.7 per cent accelerated assimilation, even where the iodine test showed little or no starch present. Ether gave similar results. The presence of varying amounts of starch in the leaves appeared to have no effect on the response, but variations in different seasons were noted. Schroeder ('07), in determining whether the retardation of respiration by hydrocyanic acid was a primary or secondary effect, used ether for purposes of comparison, and found that with the latter the reduction of respiration was gradual with small doses. With long exposure there was no recovery, as was the case with the acid. The conclusion was reached that the respiratory response to ether was of a secondary nature and resulted from injury as the primary cause.

Palladin ('10), in a study of the effect of toxins on the respiration of living and dead plant tissues, determined the carbon dioxide evolved by corms of *Gladiolus Lemoine, G. Calvilli,* bulbs of *Allium Cepa,* and seedlings of wheat and *Vicia Faba.* Various chemical agents were employed, among them ether. In general the results showed carbon dioxide stimulation by toxins, the effect of which disappeared when the plants were subjected to lethal conditions. The other phase of Palladin's work will be considered later.

Müller-Thurgau and Schneider-Orelli ('10) recorded both carbon dioxide data and sugar content of etherized potatoes previously stored at 0° C. They concluded that etherization,—at least in comparison with the effect produced by heat,—had little effect upon the metabolic processes concerned in the conversion of sugar with which carbon dioxide evolution is associated. These results are open to criticism in view of the fact that in all cases where the effect of ether was studied the tubers were halved, which introduced the important additional wound factor with respect to respiration processes. Irving ('11) has noted the difference in the effect on respiration of single doses of chloroform *versus* continuous treatment, as well as the effect of this anaesthetic on assimilation, using barley shoots or leaves of the cherry laurel. With respect to respiration she found that the effect depended upon the dosage, with a regular progression in the respiration curve between the augmentative effect of minute doses and the inhibitory action of large ones. The increased respiration effected by small amounts of vapor could be maintained if such amounts were continuous, and normal respiration was restored with its withdrawal. Medium dosage resulted in an initial outburst of carbon dioxide followed by a decline much below normal, the rate and extent of decline increasing with the dosage. With stronger doses the initial stimulus fell rapidly to zero. The early period of application was found to be the most effective time, as the same respiration curve was found to hold thereafter when chloroform was withdrawn or continued. The destruction of chlorophyll in barley leaves, with exudation of water from the stomata, and the browning of the cherry laurel tissues and decomposition of the cyanogenetic glucoside were indices of the lethal action of the anaesthetic. Interesting effects on assimilation were also noted. Minute doses which had no detectable effect in darkness arrested assimilation in the leaf in light. Recovery of assimilative powers could be noted following a short exposure to a low concentration. Quite moderate doses abolished all traces of assimilation, while larger ones acted so rapidly that

there was no difference between reactions in light and darkness.

The experiments of Hempel ('11) indicated that small ether dosages of short duration accelerate carbon dioxide production in *Pisum* seedlings, especially at low temperatures, while large doses proportionately retard such action; the after-effect of narcosis was a retardation irrespective of the concentration used. Thoday ('13) investigated the quantitative relation of carbon dioxide evolution and oxygen absorption in relation to chloroform doses, using a modified form of Bonnier and Mangin's eudiometric apparatus. He believes that the increased respiratory activity produced by different agents is not necessarily of the same nature in each case and that a substance so chemically inactive as chloroform may have a relatively simple physical effect. Fresh and starved leaves of cherry laurel (*Helianthus tuberosus*) and *Tropaeolum majus* were used. In cherry laurel a weak dosage showed a stimulation in production of both gases to similar degrees. With strong dosage disorganization was effected, accompanied by a rapid inrush of oxygen, diminished evolution of carbon dioxide, and browning of tissues. With *Helianthus* similar results were obtained, but chloroform penetrated more rapidly. *Tropaeolum* appeared to be intermediate between the other leaves in susceptibility. In many, but not all, cases a relatively greater and more augmented respiratory activity occurred in anaesthetized starved leaves, as compared with the controls. In general this work confirmed that of Miss Irving.

Von Körösy ('14) studied the effect of chloroform solutions on *Elodea* under controlled conditions of temperature, light, and carbon dioxide content of water, using the bubble method. He found a range of aqueous solutions which inhibited chlorophyll assimilation; the average concentration of such solutions was .074 per cent. At such concentration the action was reversible, the usual plant activity being resumed with a return to normal conditions. The chloroform concentration noted was the same as that with which Loeb and Wasteneys obtained their results.

Haas ('17) has recently made an investigation of the effect of various anaesthetics, including ether and chloroform, upon the respiration of *Laminaria*. The change in amount of carbon dioxide evolved was noted in terms of the change in hydrogen ion concentration of the solution in which the fronds were immersed, using indicators and buffer solutions of known hydrogen ion concentration as standards. He found that exposure to anaesthetics in concentrations sufficient to produce any effect resulted in increased respiration; a decrease below the normal amount followed this in cases where the concentration of the narcotic was sufficiently toxic, but no such decrease was noted with non-toxic concentrations.

EFFECT ON PERMEABILITY

We come now to a consideration of work tending to throw light on the initial means whereby anaesthetics effect their action on plants,—namely, the relation of anaesthesial agents to cell permeability. It is evident that the question of the theory of anaesthesia and the relations of narcotics to cell conditions affecting enzyme action must bear a close relation to this factor. The work of Overton, to be discussed later, may also be classed as studies in cell permeability.

Wächter ('05[a]), in a quantitative study of the exosmosis of reducing and non-reducing sugars in *Allium Cepa* and *Beta vulgaris* in relation to the inhibitory effect of various agents on such osmotic action, found varying effects of ether solutions on subsequent plasmolytic action after a previous treatment with other agents, a concentration of 2 per cent being definitely lethal. Herzog and Hörth ('07) subjected yeast in an evacuated desiccator to vapors of chloroform, ether, alcohol, etc., and noted a rapid liquefaction varying in rapidity with the vapor used. The liquefaction was most rapid with the water-soluble substances, with a descending scale of action to those insoluble in water. The liquefaction was explained as a coagulation of proteins in the cell by the respective vapors, with consequent extrusion of the protein solvents. The rapidity of action, which was the point especially noted, depended of course upon the permeability of

the cell membranes to the respective substances. Chiari ('09) reported that ether, chloroform, and other narcotics increased autolysis of canary's liver, and he ascribed this effect to the solvent action of such substances upon the lipoid constituents of the cell membrane; by such action, he believed, enzymes acting upon cell contents penetrated the membrane more easily. Czapek ('10), in his studies on exosmosis with reference to permeability and surface tension, found that exposure of *Echeveria* cells to chloroform for twenty-four hours produced an alteration in the permeability of the plasma membrane, resulting in the absence of certain precipitation phenomena when treated with caffein. Ether had a similar effect; short periods of narcotization up to one hour had no such action.

Lepeschkin ('11), in a study of the chemical composition of the plasma membrane, showed that substances readily soluble in oil and poorly soluble in water, of the class designated as narcotics by Overton, were held in the dispersion phase of the plasma membrane, and that a proper concentration of such compounds in the aqueous solution bounding the cells may reach a point where resulting electric changes produce a protein coagulation. This in turn affects the selective permeability of the plasma membrane. With smaller concentrations of narcotics in the external medium the chemical composition of the dispersion phase of the membrane would also be changed, so that a certain amount of narcotic would be taken up. Believing that the osmotic properties of membranes must be altered by such action, Lepeschkin ('11[a]) made experiments to determine this point. Aniline dyes, with varying solubility properties in water, chloroform, and ether, were used, and *Spirogyra* cells served as indices of permeability. Etherized algae took up the methyl dyes from aqueous solutions to a lesser degree than the controls. That this action indicated an alteration in membrane permeability was shown by an equal staining of etherized and control filaments previously killed.

Another experiment showed permeability alteration in relation to salts by the isotonic coefficient method, the plasmo-

lyzing solutions being sucrose and potassium nitrate, the indicators epidermal cells of *Tradescantia.* The results showed a consistent reduction of permeability values as the result of narcotic action; increased osmotic pressure was also noted. Ruhland ('08, '12), however, opposes Lepeschkin's findings with respect to basic dyes with the results of his own investigations, accounting for permeability of such substances on other grounds.

Osterhout ('13, '13[a]) studied the permeability of plant tissues by electrical conductivity measurements. In connection with this work he found that anaesthetics decreased the permeability of *Laminaria* tissues. In later studies ('16) he reported that such permeability was reversible, and showed a relation between anaesthetic concentration and the degree of permeability. The relative concentrations for permeability decrease corresponded closely with those effecting anaesthesia. An increase in permeability, on the other hand, was irreversible and caused permanent injury.

Merrill ('15), in an extensive study of exosmosis in response to various factors, found that both chloroform and ether in vapor form and in solution effected a marked exosmosis from the roots of *Pisum sativum,* the first-named anaesthetic having a notably greater action. Lillie ('18) has recently reported experiments with fertilized sea-urchin eggs, which show the direct effect of anaesthetics upon permeability to water.

Harvey ('17) studied the effect of anaesthetics upon the regulation of specific gravity in *Noctiluca,* and also the effect on light production. It was found that such regulatory power was not affected unless the narcotics were used in concentrations sufficient to cause irreversible changes and death of the cells; the animals could be anaesthetized, however, by certain concentrations of ether and chloroform, so that they failed to give the customary flash when subjected to a stimulus. She concluded from her experiments that the anaesthetics affect the mechanism of oxygen utilization in the cell, and not the permeability of the cell membrane for oxygen.

EFFECT ON METABOLISM

As already stated, an adequate survey of the vast field of medical literature dealing with the varied reactions of the animal mechanism to narcotics does not come within the scope of the present paper. The writer here cites only the few investigations relating most closely to plant metabolism studies which have been noted in a search of the literature dealing with the work in plant physiology.

Hegar and Kaltenbach ('70) noted a marked albuminuria following chloroform narcosis in many, but not in all, cases observed. More recently Winterstein ('02) carried out perfusion experiments with frogs to determine the relation of narcotics to metabolic action. On the assumption that there exists a direct connection with nervous response and metabolic function, his data indicate that in ether or chloroform narcosis both assimilation and dissimilation are retarded to the same degree. Failing to accept such an assumption, which, in the light of present knowledge, we are not forced to do, this relationship remains unproved.

Hawk ('04) reported that glycosuria always followed ether anaesthesia in dogs. Baldwin ('05) states that urine following anaesthesia showed higher specific gravity, was more strongly acid in reaction, and showed excretion of acetone, indicating a distinct action of anaesthetics on metabolism. The observations of Ross and McGuigan ('15) showed that the hyperglycaemia following the anaesthetizing of animals was due in greater part to the ether itself and not to asphyxia or excitement. Watanabe ('17) confirmed the glycosuria findings of Hawk by experiments with rabbits.

In the plant field one of the most important studies of metabolic action following anaesthesia has been contributed by Johannsen ('97). Since this work was published in Danish, the inaccessibility of the data to the majority of botanical students warrants a somewhat detailed abstract of the results. The object of the investigation as a whole was to determine the relative influence of anaesthetics upon the metabolism of plants in ripening and resting stages. Preliminary experiments with peas freshly picked and others gathered

some days previously were made to determine the effect of varying amounts of chloroform on the conversion of amido-nitrogen bodies to protein form. In both cases it was evident that such change was inhibited by the anaesthetic, and in proportion to the increase of the concentration employed.

Respiration experiments in all the following studies were made by aspiration of carbon dioxide-free air through the experimental chamber into an absorption apparatus. The absorption liquid was precipitated with a 5 per cent barium chloride solution, and transferred quantitatively to a volumetric flask. After complete precipitation the supernatant liquid was titrated with hydrochloric acid. Nitrogen analyses were made by the Kjeldahl method and sugar determinations by reduction of Fehling's solution.

An attempt was next made in a series of tests to determine if hydrolytic processes were associated with the metabolic condensations characteristic of the ripening process. The materials used were green peas and fresh, green elderberries. The data obtained showed that chloroform narcosis reversed the course of metabolism in ripening seeds; the narcosis not only inhibited to all appearances the elaboration of "amide" nitrogen compounds into proteins, but increased the "amide" nitrogen as well. The condensation or synthesis of the simpler sugars into polysaccharides was also inhibited, with indications of a reversal of the process.

A series of experiments with branches of *Salix acutifolia*, potatoes, and other material showed that with respect to interruption of the rest period the probable predominance of metabolic condensation processes over hydrolytic action could be altered by etherization, and the period of inactivity suspended. These experiments were outlined in detail in another publication of Johannsen and he does not here further consider them.

Etherization of *Salix* buds (in lots of 200) showed a loss in reducing sugar content and a marked gain in nitrogen, as compared with the controls. An analysis of fat, sugar, and nitrogen relations of green lupine seeds following etherization showed a slight increase of all three products directly

after a two days' exposure, with an apparent return to normal fat content and a marked increase of sugar and "amide" nitrogen two days after removal from ether. Another series of experiments with young peas of high sugar content showed a marked gain in "amide" nitrogen content and in reducing power of Fehling's solution, with a reduction in carbon dioxide evolution following a two days' exposure to ether; subsequent aëration for two days showed an increase of the gas evolved, with a slight reduction of sugar and nitrogen values; compared with the controls, the analysis after the aëration showed a distinct increase of sugar and nitrogen products as a result of etherization.

In connection with this work a comparison was made of the "ferment activity" of peas. Seeds freed from their integuments were crushed, dried *in vacuo*, and finely powdered, whereupon the amylolytic action of the powder upon a neutral starch solution (chloroform added as antiseptic) was noted after a period of incubation. The starch was precipitated with alcohol and the supernatant liquid, after filtration and evaporation of chloroform, was examined quantitatively for its reducing power on Fehling's solution. No consideration was given in this series to the possible effect of acid formation upon enzyme activity. According to the values obtained from controls, enzymic activity increased as the sugar content of the seeds decreased; etherization resulted in a marked increase of the values indicating accelerated enzyme action.

In still another experiment the extract obtained from crushed green peas after standing in water was compared with a similar extract heated at an early stage to inhibit enzyme action. Increased inversion values for sugar were notably lower in the control extract. Green peas etherized with varying dosages showed a distinct increase in "amide" nitrogen content with increased concentration of the dose, and the same was true for reducing sugars, although the general effect of ether was again shown to be a tendency towards condensation processes. At the same time, the after-effect of etherization here appeared as an inhibition of the loss of

"amide" compounds. Increased inhibition accompanied greater doses. Similar results are reported to have been obtained with barley and with bulbs of *Crocus*.

For data bearing on the effect of anaesthetics on ripe or resting organs, a series of tests were made on onion bulbs. Etherization for a protracted period had the effect of checking the loss of "amide" nitrogen compounds. *Crocus* bulbs showed a loss in reducing sugars corresponding with the length of exposure to ether; the after-effect indicated sugar accumulation much in excess of the slower increase in control bulbs. Johannsen does not account for sugar decrease during the time of narcosis. A lengthy discussion of the results, very briefly summarized elsewhere ('96), concludes the paper. With respect to protein metabolism, Johannsen considers the alternative possibilities of the action of ether directly upon enzyme action or upon the condensation processes. The theory of the effect of the anaesthetic as a stimulus converting zymogen into an active proteolytic enzyme, —as Green ('87) believed was effected by acids,—is not accepted. To Johannsen the effect upon the condensation processes seems the satisfactory explanation. According to his view, two directly opposing changes take place simultaneously in ripening and resting organs,—a condensation of "amide" substances into proteins, and the reverse. Etherization reduces or stops the first of these processes, so that the second is more effective. The absence of direct proof of this relation is, however, admitted.

For carbohydrates the conclusion is reached that weak dosages accelerated loss of sugar in ripening organs by accelerating condensation processes; medium and strong concentrations retarded and probably inhibited condensation and resulted in the increased sugar content. Where such increase occurred without etherization, the treatment strongly accelerated it. Very strong doses caused a relative reduction of sugar accumulation during and after narcosis. The general after-effect is a reversal of chemical changes, so that hydrolytic action predominates. In many organs this results in the

abolition of the rest period. A strongly increased respiration follows an ether dosage below the injurious or lethal limit.

Czapek ('97) showed that narcotization of the conducting tissues of the petioles inhibits the translocation of the organic food materials from the leaf. Soave ('99) has reported quantitative data showing the effect of ether and of chloroform upon germinating seeds of *Arachis, Cucurbita, Hordeum, Zea,* and *Pisum,* with special reference to respiration and to the metabolism of fats and proteins. He concluded that anaesthetics inhibited anabolism without suspending catabolic processes. Zaleski ('00) made a study of the changes exerted by ether on the protein content of etiolated seedlings of *Lupinus angustifolius.* From the analyses made it was concluded that in an ether atmosphere proteolysis is retarded. Other experiments with wheat seedlings indicated that ether checks loss of glucose and induces a greater transport of this sugar from the endosperm to the plant.

Further studies of the effect of etherization on the relative amounts of nitrogen compounds in the axial parts and in the cotyledons led him to conclude that in an ether atmosphere more proteins either collect in the axes, or else are there formed,—which would argue for a stimulus of protein synthesis. Zaleski's data are in some cases open to criticism, in so far as some of the values from which he drew conclusions differ by amounts within the range of experimental error. His conclusions regarding protein translocation have also been disputed by Hempel ('11) as based on insufficient evidence.

In the study of tyrosin formation in roots of *Lupinus albus* seedlings, Bertel ('02) found that crystal formation could be induced by stimulation with chloroform, ether, and other volatile organic compounds. A narcosis of 24 hours resulted in a loss of tyrosin, which disappeared entirely at the end of 3–4 days. Since these results were obtained under aseptic conditions, Bertel ascribed them to an enzyme autolysis incited by the anaesthetic. In support of this view he stated that coincident with the loss of tyrosin was the presence in

the roots of a substance which reduced ammoniacal silver nitrate solution much more actively than did the controls.

Another extensive study of the metabolic processes following etherization has been made by Hempel ('11), using seeds and seedlings of *Pisum* and *Lupinus,* buds of *Acer pseudoplatanus,* and injured potato tubers. The data reported were of a quantitative chemical nature. From a considerable amount of analytical work the following conclusions were reached:

Protein hydrolysis with simultaneous formation of "amide" compounds, occurring normally during seed germination, was retarded by narcosis in proportion to the dosage used. Very small doses produced acceleration. The after-effect of small doses was an acceleration of catabolic processes; for large doses, a retardation.

Sugar formation (estimated as glucose) was accelerated by a small or moderate dosage of short duration. Longer exposure to the same concentrations effected a retardation.

The translocation of nitrogen compounds to the shoots may possibly be affected by etherization, since a relation appears to exist between their increase and the protein hydrolysis in the cotyledons.

Etherization of buds (*Acer pseudoplatanus*) with small doses produced an increased quantity of "amides," either in light or in darkness. Larger dosage retarded protein synthesis.

A condensation process of considerable duration involving nitrogen compounds characterizes wounding of potato tubers; such condensation appeared to be retarded by moderate ether doses. A long exposure to the ether (3 days) effected a retardation of 2 days. Large dosages inhibited the formation of "wound cork."

Hempel divides ether narcosis into three phases:

1. The excitation phase resulting from short exposure to small doses. The usual effect is acceleration of metabolism.

2. Narcosis proper, resulting from short exposure to large doses or long exposure to small ones. The usual effect is

retardation of metabolic and dependent processes, such as growth and germination.

3. The toxic phase, resulting from prolonged exposure to large doses.

EFFECT ON ENZYME ACTION

The preceding topic has considered the effect of etherization on the metabolic processes induced by enzyme action. Another phase of the subject that has engaged the attention of investigators is the direct effect of narcotics upon the catalytic agent as studied in the laboratory. Müntz ('75) erroneously attempted to distinguish between organic and inorganic fermentation phenomena by a difference in their behavior with respect to chloroform,—the latter inhibiting organic but not inorganic fermentation (". . . sans influence sur les fermentations d'ordre chimique"). Several decades later Detmer ('81) published a study of the effect of various substances on plant cells and their ferments; diastase action was studied in relation to its inhibition by various concentrations of solutions. Chloroformed seedlings were killed without inhibition of diastatic activity. In relation to the effect of external agents on enzyme action Detmer postulated four possible conditions:

1. Neither cell life nor enzyme activity is affected.
2. Cells may be killed and enzyme activity inhibited.
3. Cells may be killed and enzyme activity continued.
4. Cells may not be killed and enzyme activity inhibited.

His results led him to the conclusion that chloroform, among other substances, affected the protoplasm without bringing a direct chemical factor into play.

Bertels ('92) reported that preparations of commercial pepsin suffered injury when subjected to the action of chloroform, while digestive extracts from freshly prepared mucosa of the pig's stomach showed no such effect. Ether and chloroform, according to Fermi and Pernossi ('94), had different effects on trypsin and pepsin preparations; in chloroform they withstood a temperature of 80 degrees for 1 hour, while in ether they suffered injury. Lintner and

Kröber ('95) noted a marked retardation of enzyme activity when chloroform was added to a mixture of maltose solution and yeast extract, less dextrose resulting in the same intervals from the etherized mixtures than from the controls.

The studies of Puriewitsch ('97) were primarily concerned with the translocation of reserve food with relation to factors regulating osmotic action, but in connection therewith he noted that the presence of ether or chloroform caused inhibition of reserve-stuff movement, such movement being directly dependent on enzyme activity.

Kaufmann ('03) found that trypsin solutions at concentrations greater than 2 per cent were not injured by a 24-hour action of chloroform or toluol, while weaker ones were affected, as shown by action on gelatin and on albumin and by decrease in enzyme concentration. Grober ('04) reported the injurious action of toluol and chloroform upon the enzymes in urine. Among several factors of environment and chemical content influencing the diastatic action in higher plants Eisenberg ('07) found that ether exerted a considerable influence. Doses of 1–2 cc. per 3½ liters volume exerted an injurious action on the enzyme, and all concentrations used showed inhibitory effect. Butkewitsch ('08) noted that toluol and chloroform induced starch hydrolysis in bark and cortical tissues of twigs of *Morus alba* and *Sophora japonica.* Both the bark and an aqueous extract thereof inverted maltose, but an alcoholic extract did not. The effect of the antiseptics is regarded as analogous to that of low temperature in reducing the activity of a starch-synthesizing enzyme.

Carlson and Ryan ('08) showed that increase of diastatic concentration in the saliva of the cat resulted from general ether anaesthesia, while Carlson and Luckhardt ('08) found under like conditions there resulted a slight decrease in the blood diastase of animals. Apsit and Gain ('09) reported that wheat seedlings killed by sulphuric ether retained their diastatic activities. Müller-Thurgau and Schneider-Orelli ('10) ascribed to ether but little action on the processes concerned in the conversion of sugar in stored potatoes. Howard, in his periodicity studies already referred

to ('15c), attempted to collect data upon the effect of etherization on the enzymic activities of his material, with respect to diastatic, proteolytic, lipolytic, and oxidative action. His results, however, were all of a general and qualitative nature, no chemically quantitative data being presented. In line with Carlson, Ross and McGuigan ('15) showed that ether anaesthesia does not increase the diastatic power of the blood serum. Watanabe ('17) found the diastase of rabbit's blood to remain practically constant except for a slight tendency to increase immediately after the anaesthesia. Burge ('17) noted that the catalase content of the blood decreased during ether administration and increased during recovery from the anaesthetic. The decrease, he believed, may be the cause of decreased oxidation during anaesthesia. Catalase action was destroyed *in vitro* by exposure to ether vapor, as during *in vivo* conditions. In such case it was not restored to normal amount when the ether was removed by bubbling air or oxygen through the blood, as occurred *in vivo*.

EFFECT ON CHEMICAL REACTIONS

The effect of anaesthetics on chemical reactions can hardly be considered as a phase of the present subject distinct from permeability or metabolic response. It will, nevertheless, be so reviewed here because of the striking and apparently direct relation between the chemical products noted and the anaesthesial stimulus to enzyme action.

Mirande ('09) was the first to report the very quick response of the leaves of *Prunus Laurocerasus* to the action of ether and other stimuli by liberation of hydrocyanic acid. This reaction was indicated by the sodium picrate paper test. Plants of several other genera responded in a similar manner. Guignard ('09) followed this work with an account which showed that the chemical reactions involved the hydrolysis of a cyanogenetic glucoside, which hydrolysis is effected by an alteration of cell permeability.

Vinson ('09), in a preliminary note, indicated that the fruit of the date palm when subjected to the vapor of acetic

acid for 12–15 hours underwent a marked acceleration of the ripening process. Waller ('10) concluded after a quantitative colorimetric study of the Mirande-Guignard hydrocyanic acid phenomenon that the evolution of the acid, in relation to electrical response as an index of life, was a post-mortem result. This, in view of the rapidity with which acid production has been noted, argues for an extremely rapid action of the anaesthetic on permeability. In later papers Vinson ('10, '10[a]) showed that the fore-mentioned forced ripening of dates could be effected by a great variety of substances, volatile and liquid. In general, the more volatile the substance the quicker the action. It appeared from the results that the chemical structure of the stimulants was not a factor, but that the stimulus was due to the killing of the protoplasm by penetration of the stimulant, with consequent release of previously insoluble intracellular enzymes. This explanation was supported by heating dates to varying degrees. A temperature that killed the cellular protoplasm without injuring the enzymes effected ripening. That ripening depended upon the enzyme relation or condition appeared evident from the fact that, despite the presence of considerable invertase, the sucrose of green dates was very slowly inverted; if, however, the fruit cells were crushed by grinding, rapid inversion resulted. It is believed that by the death of the protoplasm by toxic, thermal, or mechanical means, ripening is facilitated by the conversion into soluble form of an enzyme previously held in insoluble condition by the living protoplasm.

Heckel ('09) reported the action of anaesthetics in rapidly liberating cumarin from leaves of *Liatris spicata, Angraecum,* and other plants, and melilotol from *Melilotus officinalis,* the phenomena being accompanied by plasmolytic changes. In a later communication ('10) he announced that chloroform and ether both accelerated vanillin formation in *Vanilla planifolia* by causing excretion of water and hastening the drying process.

Results of a most striking nature with special bearing on the writer's problem have been announced by Willaman ('17), who studied the effect of anaesthetics upon the cyanogenetic

content of *Sorghum vulgare*. He was able to show an increased yield of hydrocyanic acid, both glucosidic and non-glucosidic, from leaves exposed to chloroform and ether vapors. This would indicate a stimulation of both hydrolytic and synthetic enzymes and is regarded by Willaman as a demonstration of enzyme synthesis *in vivo*. In this connection he also reported that the enzyme powder extracted from chloroformed leaves was 25 times as active towards an amygdalin substrate as the enzyme from controls.

STIMULATION, INHIBITION, AND THE THEORY OF NARCOSIS[1]

Any consideration of the theory of narcosis or any attempt to explain the nature of stimulation involves a discussion of the structure of the plasma membrane and the theories of its composition and permeability.

Probably the first theory of anaesthesia was that essayed by Claude Bernard ('78), who distinguished between anaesthetics and narcotics, a distinction which, in the light of present knowledge, is no longer accepted. As anaesthetics he classified substances such as chloroform and ether which acted on both plants and animals and whose action was temporary. Under narcotics he grouped those which did not affect all protoplasts but only nerve ganglia. The present understanding of stimulation and toxic action of substances in the light of their physico-chemical behavior no longer regards any distinction between narcotics and anaesthetics. Largely on the basis of his study of the effect of anaesthetics on nerve tissues, Bernard regarded anaesthesia as the result of a coagulation of the protoplasm, with the resumption of normal conditions by elimination of the poison from the tissue.

Dubois ('83) advanced another theory,—that of dehydration of the tissues by anaesthetics,—as the result of his experiments with plants, and Verworn ('00) considered the action to be due to an inhibition of processes dependent upon the presence of oxygen. This theory has not been universally accepted, and the work of some investigators tends to show

[1] For an excellent discussion of this subject and of the related literature see Lillie ('16).

that it is untenable. Thus, Loeb and Wasteneys ('13, '13[a]) have reported that chloroform, among other narcotics, produced complete narcosis in fertilized eggs of the sea-urchin, without practically lowering the rate of oxidation; similar results were obtained with fish embryos and with medusae.

Modern theories of narcosis, based on studies of membrane permeability, structure, and composition, have been expounded since the well-known work of Overton ('95). This investigator published data on the osmotic properties of plant and animal cells, in which he adduced evidence to show that although the permeability of solutions towards protoplasm decreased with their specific gravity, the size of the molecule was not the sole conditioning factor. This work was the forerunner of his study of narcotics ('01) in which he reached conclusions previously arrived at independently by Meyer ('99) and which form the basis for the much-disputed Meyer-Overton theory of narcosis. According thereto the conclusions are:

1. All chemically "indifferent" compounds which are lipoid solvents act as narcotics on protoplasm wherever they come into intimate contact therewith.

2. Action is effected first and most strongly in cells in whose chemical composition lipoids predominate,—hence especially in nerve cells.

3. The relative effectiveness of such narcotics is dependent upon their mechanical affinity for lipoids, on the one hand, and for the other cell constituents, especially water, on the other. It is determined in a mixture of water-soluble and lipoid constituents upon the partition coefficient; i. e., the effect varies directly with increase in lipoid solubility.

Lepeschkin ('11) essayed to throw light on the chemical nature of the plasma membrane and of the dispersion medium of the outer protoplasmic layers, wherein he considered osmotic selective power to reside. This he attempted by a comparison of permeability changes. Comparisons were made of the concentrations of various solutions sufficient for albumin coagulation with those necessary for the complete coagulation of membranes of *Tradescantia, Spirogyra,* and

Saccharomyces cerevisiae. Coagulation temperature limits of the membranes were noted and compared with those of proteins. A determination of albumin coagulation concentrations for lipoid solvents, as ether, chloroform, etc., showed that they were much greater than those acting on the plasma membrane. The concentrations appeared to correlate with the partition coefficients in water and oil. The general conclusion was reached that proteins or loose protein combinations are important constituents of the plasma membrane, and that bodies of a lipoid nature are also present; whether the latter are lecithin or cholesterin compounds, as Overton has suggested, or merely fat bodies, was not concluded. Data harmonizing with the Overton hypothesis have been published by Zehl ('08), who studied the action of varying temperatures in relation to the toxic action of a diversity of poisons upon two fungi. The noteworthy results with respect to the present discussion were the marked reductions in toxicity of the common anaesthetics for both *Aspergillus* and *Penicillium* with rise in temperature, the limiting toxic concentrations of both chloroform and ether being notably increased. Such action, Zehl has pointed out, accords with reduction of the partition coefficients and threshold concentrations of narcotics with increase of temperature, as shown by Overton.

Choquard ('13), from a study of muscular response of tissues with scant and abundant lipoid content to treatment with narcotics, found the Overton theory insufficient explanation for the results obtained. Rapidity of narcotic penetration appeared to be an important factor involved. Another type of experimental data is that of Alexander and Cserna ('13) who studied the gas exchange of the brain in ether narcosis as compared with the narcosis produced by non-lipoid solvents. The values of the carbon dioxide production and oxygen evolution associated with the respective kind of anaesthesia are the grounds on which these workers take exception to the Overton theory as an explanation. They incline to a view more in harmony with that of Mansfeld ('09). Osterhout ('13[a]) also opposes the Overton view with the results of his experiments on plasmolysis of *Spirogyra* with

solutions of inorganic salts. Traube ('13) regards lipoid solubility as an accompanying phenomenon, and not the cause of narcotic action. According to his theory, the action of anaesthetics is caused not by their solution in the cell lipoids, but by adsorption and surface-condensation of these substances at physiologically active surfaces; such surfaces may be of lipoid or protein nature. The action of narcotics, according to this view, is due to a lowering of the surface-tension rather than to lipoid solubility, such tension changes affecting the degree of adsorption and hence of narcotic action.

Lillie ('09, '09[a], '09[b], '11, '12, '12[a], '13, '13[a], '13[b], '14, '16, '18) has devoted much attention to the effect of anaesthetics on the plasma membrane, working especially on the larvae of *Arenicola* and eggs of *Arbacia*. These contain a pigment, which, under proper osmotic conditions, passes into the surrounding medium. Proper solutions of electrolytes thus caused exosmosis, but the addition of certain narcotics to the same concentrations inhibited such action to varying degrees. In general, all anaesthetics were markedly injurious in strong concentrations, while in weaker ones they showed a protective action against the electrolyte. According to Lillie the solubility relations between the lipoids of the membrane and the narcotics result in a reduction of permeability produced by increase in volume of the lipoid particles. The essential effect, according to Lillie, is a modification by narcotics of the physical properties of the plasma membrane of such nature that the latter, under the usual conditions of stimulation, fails to undergo the increase of permeability essential to such stimulus. A real stimulation must therefore involve a well-defined increase of membrane permeability.

The outstanding feature of the literature here surveyed is the variation in the effect produced by the same narcotic agent. Whatever the manifestation of activity in plant or animal that has been studied, the consensus of results points to a condition of stimulation, inhibition, injury, or death, varying with the concentration used or the length of exposure. The theory advanced by Lillie appears to furnish an explana-

tion for such different reactions. The permeability changes in the plasma membrane produced by narcotics involve differences in ion concentration on the two sides of the membrane, with resulting differences in electrical potential. It may be assumed that the cations concerned in the production of this potential are the ions derived from dissociation of carbonic and other weak acids produced in metabolism, and that carbonic acid is the chief electrolyte concerned in the production of this potential. In other words, the plasma membrane of the resting cell may be regarded as the seat of a potential difference and is electrically polarized in such a way that the solution in contact with its outer face is positive with respect to the enclosed protoplasm and that during stimulation this potential difference increases. If this Nernst theory of cell polarization is accepted, any increase in the ionic permeability of the membrane produces a corresponding change in its polarization; the phenomena involved in such electrical changes are hence primarily responsible for stimulation.

Lillie points out that the most evident chemical effect of muscle stimulation is increased carbon dioxide production, and his explanation of this increase,—which may be applied to plant tissues,—is that it is due to the increased permeability of stimulation. Chemical equilibrium, it is recognized, depends upon equalization in velocity of the opposite pairs of chemical reactions. If the reaction products of one side of the equation are removed, acceleration results. If such products are slowly but continuously removed the relative velocity of the reaction producing them will depend upon the rate of such removal; any increase in this rate of removal from a system of interacting compounds in an approximate equilibrium will result in a corresponding acceleration of the process in the direction of the removed substance.

Now, according to Lillie's view, carbon dioxide is the reaction product whose rate of removal from the cell determines the velocity of the chemical processes concerned in stimulation. Normally the rate of removal is controlled by the degree of permeability of the plasma membrane. A slight in-

crease in the permeability, induced by the solvent action of an anaesthetic on the membrane lipoids, produces stimulation; an increased amount of such lipoid solvent makes a change in membrane permeability difficult, with resulting inhibition or narcosis. A still greater amount injures the membrane, causing irreversible cell changes and consequent death.

THEORY OF ANAESTHETIC ACTION ON ENZYMES

A consideration of the possible mode of action of anaesthetic substances upon enzymes seems desirable, inasmuch as such action involves the effect upon the catalytic agents of cell metabolism, as distinct from the direct effect upon plasma permeability. Palladin ('10), in a paper already noted, pointed out four possibilities in his study of respiratory enzymes. Regarding this group of catalysts these possibilities are:

1. Direct effect on one or all respiratory enzymes.
    a. Stimulation as a catalyser.
    b. Inhibition as an anti-ferment or toxin.
2. Effect on reactions which precede enzyme formation.
    a. Stimulation by conversion of zymogens to enzymes.
    b. Inhibition by killing of zymogens.
3. Effect on reactions which furnish material for respiration (i. e., for cleavage and oxidation).
    a. Stimulation as catalysers for proteolytic and glucosidic enzymes.
    b. Inhibition as an anti-ferment or toxin.
4. Effect on the environment of the enzyme, with resulting increase or decrease of enzyme activity.

Palladin's experiments led him to the general conclusion that respiratory stimulation depended on the increased conversion of zymogens to enzymes. Such increase, however, he considered, was accompanied by increased enzyme destruction, by which explanation he accounted for the equal amounts of enzyme in control and in dead plants.

Armstrong and Armstrong ('10) reported studies on hydrolysis of the glucoside prulaurasin in the cherry laurel, which were inspired by the earlier reports of Mirande ('09)

and Guignard ('09). The stimulative action of various volatile substances was noted, as well as the effect of solutions of electrolytes and non-electrolytes. Observations showed that all substances which were able to penetrate the cell membrane acted as stimulants to varying degrees. The most active stimulants were those with little, if any, chemical activity, and which have very slight attraction for water,—i. e., they are anhydrophilic. It was also shown that hydrogen cyanide or benzaldehyde,—two of the products of prulaurasin hydrolysis,—had a cyanogenetic effect upon the leaves similar to that of the other vapors studied; hence the Armstrongs concluded that the action of the penetrating substances could not be primarily a chemical one, since it could be effected by some of the products resulting from the hydrolysis itself. The stimulation produced by chemically inactive vapors is ascribed to their action in causing changes in the concentration of the cell solution of such nature that the glucoside and the enzyme are brought into contact under conditions which promote hydrolysis. All substances which enter the cell effect such concentration changes to some degree. In the case of substances in solution the water entering with the solute is probably a factor in altering the concentration of the cell fluids. It is evident that this theory is based upon the assumption of a purely "mechanical" activity on the part of the stimulating agents, as a result of which the concentration is lowered to a point which is either favorable to a change previously existing only in a potential state, or is more favorable to an action already occurring.

The effect of the same stimulating substance acting as a narcotic or lethal agent is ascribed to changes in the osmotic pressure. Assuming an active cell, the pressure varies continually as carbon dioxide and other simple compounds are removed from solution and combined in more complex forms. With their disappearance others diffuse in from without. The more complex molecules are in part more or less inactive by incorporation in the protoplasmic mass, but some have a marked attraction for water. The products of the "downgrade" changes which occur at the time of synthetic trans-

formations are also hydrophilous. The result is that the water in the cells is in a constant state of dissociation between the form $(H_2O)_x \rightleftharpoons X(H_2O)$. Protoplasmic movement and the associated changes in cell contents are probably dependent on the exchanges occurring between the "hydrolated" colloid surfaces and the solution. The action of the anaesthetic in increased concentrations is believed to cause stoppage of movement by increased osmotic tension, which produces a balance of the attraction between the protoplasm and the solution. In a later communication ('11) these authors point out the applicability of Starling's name of hormones, or excitants of functional activity to the anhydrophylic stimulants above noted, which pass through differential septa.

From a general consideration of the foregoing survey of literature it is clear that the investigations on plants group themselves into three fairly distinct classes. In the first class are all those concerned with streaming, tropisms, germination, growth, transpiration, respiration, etc., in which the activity noted, whether physical or chemical in nature, may be regarded as the "end product" of the etherization stimulus. In the second group are the studies dealing with the more immediate effect upon metabolism proper. The third comprises those relating to the effect on membrane permeability, upon which all the other phenomena undoubtedly depend.

For the moment we are more especially concerned with the metabolism experiments. These have been of great interest and value, not only for the light which they throw upon the chemical processes going on within the plant at different stages of its life, but also for the impetus they furnish to pursue further the question of enzyme relation to stimulation phenomena.

If it be granted that the activities studied in experiments of the first group,—those of ultimate response,—are primarily dependent upon the fundamental metabolic processes within the plant, we are led to one of two conclusions: (1) The effect of narcotics is one solely concerned with membrane permeability. All subsequent effects on metabolism are the indirect results of such permeability changes. (2) The effect

of narcotics may be one directly involving the activation or inhibition of the enzymes of metabolism.

In either case the effect of anaesthetics upon enzyme action is deserving of study. For if the second hypothesis be discarded at once, there still remains the very evident possibility that the permeability changes induced in the membranes by narcotics (and such changes are established facts) may, by altering the conditions of concentration within the cell, produce changes in the chemical condition or activity of the enzymes.

Johannsen ('97) does not regard the conversion of proenzymes or zymogens to active forms as a plausible explanation of his metabolism studies, and although he admits the possibility of increased enzyme activity following stimulation, he believes it doubtful. His explanation lays all the emphasis upon the condensation processes and their reversal. We cannot, however, escape the fact that, so far as we know, enzymes are responsible for such syntheses and hydrolyses; therefore they have either been incited to action or inhibited by concentration changes, or else they have been directly acted upon,—converted from inactive to active form,—or the reverse.

On the other hand, as we have noted, Palladin ('10) in his studies of respiration enzymes not only considers the stimulation or inhibition of existing enzymes, or the conversion of zymogens to active forms (or the reverse) as possible, but actually concludes from his experiments that respiratory stimulation depends upon increased zymogen conversion. In addition we have the very striking results of Willaman ('17) which indicate the possibility of enzyme synthesis *in vivo* and the still more remarkable increase in the activity of an extracted plant enzyme as the result of narcotization.

In view of the contrasting viewpoints, it has seemed desirable to attempt to secure additional data bearing on these points by studying the effect of ether upon plant enzymes, as measured by their action upon various substrates under control conditions, using quantitative chemical methods.

It must, of course, be here emphasized that such data cannot in themselves be considered as quantitative. The difficulties and errors involved in the methods and procedure of enzyme extraction under the most favorable conditions preclude the consideration of resulting data as other than comparative. With this limitation, however, they should, whether of positive or negative nature, nevertheless prove of interest.

Methods and Materials

For the control of temperature conditions a large constant temperature water bath of 85 liters capacity was devised. Details of construction of the apparatus will be reserved for future publication. It will suffice to say that the water container was a rectangular galvanized iron tank 112 cm. long, 36 cm. wide, and 41 cm. deep, insulated with solidly packed excelsior. The reservoir permitted the use of two parallel rows of six two-quart glass Mason jars which served as containers of the material studied. Heat maintenance and control were electrical. Uniformity of temperature in the bath was insured by two electrically driven paddles of the propeller type. An indicator arm with pen attached, similar to the recorder of a Friez thermograph, was connected with the heat-controlling device and registered temperature deviations on the dial of a clockwork recorder. With preliminary trial and adjustment it was found possible to maintain a desired temperature within $\frac{1}{4}$–$\frac{1}{2}$° C.

The plant material studied was in all cases brushed and washed in tap water, disinfected by immersion for two or more hours in a 0.1 per cent solution of mercuric bichloride, washed for several hours in running water, and finally dipped in two changes of distilled water and one of 70 per cent alcohol, when it was turned out on clean absorbent paper and when dry placed in the jars. Each lot was weighed and transferred to a numbered cheese-cloth bag before disinfection, thus reducing handling to a minimum.

At the beginning of an experiment the control portions and those to be etherized were placed in the water bath at the

desired temperature and subjected to a preliminary heating for 24 hours to insure a uniform heat throughout the tissues. The jars to be etherized were then fitted tightly with rubber stoppers, through each of which passed a piece of glass tubing of about 5 mm. diameter and 8 cm. in length. The upper end of this tube was fitted with rubber tubing and a screw clamp. From the part below the stopper a small basket of copper gauze containing a tuft of cotton was suspended by copper wires. Before etherization the stoppers were forced in as tightly as possible, and the juncture of stopper and jar, as well as the surface of the former where the tube entered, was thoroughly sealed with a liquid mixture of equal parts of beeswax and paraffin. The calculated ether dose was dropped with a pipette through the tube on the cotton below, and the screw clamp immediately and very securely tightened. That no ether escaped during the experiment, even with the pressure resulting from the temperatures used, was evident upon opening the clamp at the end of the exposure. The control jars were also fitted with rubber stoppers, but were not sealed. In every case the dosage used per jar was 1 cc. of Merck's ether for anaesthesia. Johannsen ('06) in his forcing experiments recommends 0.4 gm. per liter volume, and the amount used in the following work approximates this very closely on the basis of 1800 cc. volume per jar.

Upon removal from the jars at the close of this part of an experiment, the plant extracts, whether studied as such or used for enzyme extraction, were made in the following manner: The bulbs or corms were rapidly grated on a fine-meshed, flat grater into a large porcelain dish and transferred in a manner as nearly quantitative as possible with a minimum amount of distilled water to water-tight, tin cylinders 15 cm. high and 6.5 cm. in diameter, with tightly fitting covers, which were at once placed in a freezing mixture of ice and salt. At the end of a sufficient time the frozen cylindrical mass was removed from each tin and again grated. The resulting mass of snow was then transferred to large glass jars for extraction. In the case of the preliminary experiments with tulips, where the extracts were studied directly, extraction was made

by addition of distilled water to a volume of 750 cc., and the liquid finally obtained after straining and filtering through a Buchner funnel made up to 1 liter, plus 1 per cent toluol as antiseptic.

In the preparation of the enzyme powder the melted mass was extracted for 18 hours with three times its volume of distilled water, plus 1 per cent chloroform, after which it was strained and then filtered through a Buchner funnel. The solid residue was ground for 20 minutes with an equal volume of fine quartz sand and further extracted for 24 hours with 2 volumes of water, after which it was likewise strained under pressure and filtered. The final solutions thus obtained were of a heavy colloidal nature. By this freezing and grating method, with subsequent grinding and secondary extraction, it is believed that the plant cells are thoroughly ruptured and that the extraction is likely to be as complete as it is possible to make it without special apparatus for comminution and pressure. The tissue pulp after grinding showed under the microscope a very thorough disruption of the cells. The colloidal nature of the solutions made their filtration under pressure a slow process, so that during it, as well as during the time necessary for the later filtration of the enzyme precipitate, all solutions were preserved with 1 per cent toluol.

Precipitation of the enzyme-protein complex was effected with 95 per cent alcohol, in the proportion of three volumes alcohol to one of plant extract. The precipitate obtained after treatment for fifteen minutes was collected under pressure upon filter paper, and quickly dried with an electric fan at room temperature, after which the papers were stored in glass-stoppered bottles for future use. Later in dissolving the dry enzyme material it was found that the greater portion on each filter was easily removed by moistening it and scraping the surface with a safety razor blade. A moist chamber or any vessel with a flat bottom was found satisfactory for the work. The residue on the filters was obtained after trituration in a mortar, by solution in water, and straining through cloth.

Experimental

A preliminary experiment was made with tulip bulbs for the purpose of obtaining data on optimum conditions of temperature and exposure with respect to etherization. The bulbs were freed of their papery integuments without injury to the scales and etherized after disinfection, as already outlined. In one series a temperature of 25°C. was maintained and in the other 35°C. At each temperature exposures to ether of 12, 24, and 36 hours were made, with corresponding controls. The method of obtaining the plant extracts for the analyses has already been stated. After extraction the solutions were heated as rapidly as possible to 90°C. in the hope of inhibiting further action of enzymes present and, upon cooling, made up to liter volume. Analyses of aliquot parts were then made for content of glucose, sucrose, and maltose, using the modified Bertrand method of Shaffer ('14). For the maltose determinations hydrolysis was effected with 5 cc. concentrated hydrochloric acid plus 50 cc. distilled water per 20 cc. extract. All portions were simultaneously heated for 1½ hours at 100° C. in an Arnold sterilizer, after which they were neutralized to phenolphthalein with 20 per cent sodium hydroxide and made up to 100 cc. volume. Ten-cc. portions were then taken for sugar determinations.

In making determinations of sucrose content based upon invert sugar values, a modification of the order of procedure in the Shaffer method was necessary. It was found that if inversion were attempted with 10 per cent citric acid, as suggested by Davis and Daish ('13), and the Shaffer method then used, that it was impossible to centrifuge out the colloids precipitated by addition of the dialyzed iron. It is possible that this is due to a solution of the iron in some citrate combination analogous to solution of copper in Fehling's solution. To avoid this difficulty, the proteins were first precipitated from equal portions of each solution by the same method and then inverted with citric acid by exposure to boiling temperature in the Arnold sterilizer for 15 minutes. The usual neutralization with alkali and increase to standard volume followed, after which the balance of the Shaffer method was continued,

with due allowance in the final computations for the difference in concentration of sample.

The numerical values of the resulting sugar determinations are not presented, for the reason that they show quite convincingly that the carbohydrate enzymes were not inactivated by heating to 90° C. The maltase values, for example, showed a content of that sugar which ranged from 14 to 41 per cent of the fresh weight of the bulbs. The findings for sucrose showed no such disproportionate values, neither did they afford any data bearing on optimum conditions of temperature or length of etherization. The only point of value that appeared was the fact that the sugar in the bulbs was not present to any extent in the form of glucose, but probably all as maltose. The very great sugar formation must be ascribed to a continued action of the carbohydrases following the heating to 90° C. until the time of analysis. This period, which involved the time necessary for completing one of the series and the subsequent extraction process, was of considerable extent.

It would appear from these results that the accurate determination of sugar values or analyses of similar nature from solutions thus obtained are not practicable, since a temperature sufficiently great to inactivate the enzymes present would, in the time necessary for its application, undoubtedly affect other hydrolytic changes in the presence of organic acids which would also introduce a large factor of error. Apparently the only satisfactory means of dealing with plant extracts for analytic purposes is that of alcohol extraction *in vacuo* similar to that described by Davis and Daish ('13, '16). In the absence of any definite conclusions from these preliminary trials, it was decided to use the mean conditions of temperature and time of etherization in the experiments in which the extracted enzymes were to be tested. Accordingly the temperature used was 30° C. and the exposure to ether 24 hours. The experiment was divided into two sections; in the first the material was extracted immediately at the close of the etherization period and in the second it was aired for 18 hours after etherization and then extracted.

For this study corms of a hybrid *Gladiolus* (*G. gandavensis* × *G. psittacinus*) were used. These were stripped of their coarse outer scales and the basal parts carefully scraped clean, without injury to the living tissues. They were sorted for uniformity of size and condition and divided into four lots of 20 corms each. There was no evidence that the corms had started active growth, but it may be assumed that at the time they were in the last stages of the resting period. The weights of the several lots and their treatment follow:

Lot	No. of corms	Weight in gms.	Treatment
A	20	403.2	Etherized for 24 hours and extracted.
B	20	403.2	Control.
C	20	402.9	Etherized for 24 hours and aired for 18 hours before extraction.
D	20	401.8	Control.

Two smaller lots, C and D, of 11 corms each, weighing respectively 191.7 gms. and 194.3 gms. were also sorted at this time, and after disinfection placed in cold storage for future experiments on catalase action.

The following substrates were used for a determination of the action of the respective enzymes:

Starch (Merck) Dextrin (Merck)	for amylases
Sucrose (A. Daigger & Co., h. p.) Maltose (Merck) Inulin (Merck)	for sucrases
Ethyl acetate (Sargent & Co., c. p.) Ethyl butyrate (Sargent & Co., c. p.) Olive oil (Merck) as emulsion	for lipases
Asparagin (Merck) Acetamid (Merck)	for amidases
Albumin (Merck) Casein (Baker) Peptone (Bacto-peptone) Albumin (Merck) in digestion tubes	for proteases

The substrates were all made up in 1 per cent aqueous solutions with the exception of the starch solution and the olive oil emulsion. Casein was dissolved in sodium hydrate and then made up to volume with water. The preparation of the starch and oil substrates has been outlined by Zeller ('16), but the details are here repeated for those to whom his paper may not be accessible.

Five grams of soluble starch were added to 300 cc. of distilled water and while constantly stirred brought to boiling. This was added to a flask of two liters capacity containing 1200 cc. of hot distilled water, and the contents then boiled with a reflux condenser for 2 hours; when cool the solution was made up to 2 liters, plus 1 per cent toluol as antiseptic. The above constitutes what is known as a $\frac{1}{4}$ per cent solution.

The method for making the olive oil emulsion is one which Davis ('15) and Zeller ('16) ascribe to Bloor, but the writer has been able to find no description of it by the latter in the literature, and it is therefore taken from the sources indicated. The procedure was as follows: Twenty cc. olive oil were dissolved in hot absolute alcohol. This was placed in a hot funnel, the tip of which had been drawn out to a very fine bore, and the hot solution of oil in alcohol passed drop by drop into 200 cc. of cold distilled water which was vigorously stirred throughout the process. The resulting milky emulsion was then boiled to expel the alcohol and upon cooling made up to 1 liter with distilled water.

The extracted enzymes of the first series (lots A and B) were dispersed in such a volume of doubly distilled water that 0.8 cc. of solution represented 1 gm. of fresh tissue. In the second series (lots C and D), loss by accident of one-half the dispersion just previous to use necessitated its dilution to half the strength of that in the first series. Ten cc. of dispersion in each case were added to 50 cc. of substrate in Erlenmeyer flasks of 125 cc. capacity, with 0.5 cc. toluol as antiseptic. For controls 10 cc. of distilled water per flask of substrate were substituted for the volume of enzyme. Comparison was also made between active and inactivated enzymes by a parallel series of substrate flasks containing equal

volumes of the enzyme dispersion previously autoclaved at a pressure of 15 pounds.

The sucrose substrate was acidified with 0.1 cc. of decinormal hydrochloric acid, since invertase is known to act best in acid medium. The solutions of albumin, casein, and peptone were made neutral, and for the alkaline series of these substrates 2 cc. decinormal sodium hydrate added to each flask, with an equal volume of water to the neutral ones. The digestion tubes were made by coagulation in hot water of a 10 per cent aqueous solution of albumin in glass tubing of 2 mm. diameter. These tubes were placed in 50 cc. of distilled water plus 0.5 cc. toluol, and 2 cc. decinormal hydrochloric acid or sodium hydrate added to the acid and alkaline series respectively. All flasks were placed in the incubator and maintained at 40–41° C. throughout the several periods of incubation. At the close of such periods they were placed in the autoclave, subjected to 15 pounds pressure for a few minutes, and upon cooling were used in the quantitative determinations hereinafter discussed.

The period of incubation varied with the substrate studied. The time for checking enzyme action was approximately determined by the use of trial flasks of substrate with enzyme extract from the etherized series. Such flasks were used only for the carbohydrates. In the case of starch, drops from the trial flask were tested on a spot-plate with iodine solution at intervals after the beginning of incubation until the end point appeared to be approaching; at this point the flasks were autoclaved. For dextrin, sucrose, maltose, and inulin, 10-cc. samples were taken from similar test flasks at one-half or one hour intervals, and the relative amounts of cuprous oxide precipitate obtained with Fehling's solution were noted. On this basis the carbohydrates were incubated for the following periods: starch, 45 minutes; dextrin and maltose, 5 hours; sucrose and inulin, 9 hours. The time of incubation for all the other flasks in the experiment,—31 days,—was purely arbitrary, and was based on the extremely slow action of enzymes other than carbohydrases, as observed in previous work in this laboratory.

As in the preliminary experiments already noted, action on carbohydrates was determined in terms of conversion to glucose. In the absence of inversion processes, the Shaffer method adapted for plant analysis, as outlined by Davis ('15), was used. For lipase action 10 cc. from each flask of ester substrate and of oil emulsion were titrated with phenolphthalein against decinormal sodium hydrate. For determining the conversion of amido- and amino-nitrogen into ammonia by the enzymes acting on acetamid and asparagin, the simple and rapid colorimetric method involving the use of "Permutit," recently announced by Folin and Bell ('17), was employed with Kober's modified Dubosc colorimeter. The action of proteolytic enzymes upon their respective substrates was noted in terms of amino-nitrogen split off, using the Van Slyke ('12) "micro" apparatus and 2 cc. of each substrate. Action upon the coagulated albumin in the digestion tubes could of course be estimated only in a general way because of the irregular masses remaining at the close of the experiment.

For the catalase experiments the two lots of *Gladiolus* of 11 corms each, already noted, were employed, after the usual preliminary heating to 30° C., one lot being etherized for 24 hours at that temperature, the other serving as a control. At the close of the etherization period the catalase extract was prepared from both lots of corms by the method outlined by Appleman ('10). In order to eliminate a possible factor of error in catalase determinations caused by differences of time involved in preparation of two lots of extract, both lots of corms were, with the aid of an assistant, treated simultaneously throughout all the processes of enzyme extraction. In a similar manner simultaneous comparison of the action of the two enzyme extracts upon the peroxide solution was made by the use of two sets of apparatus and two observers. After removal from the jars the corms were immediately grated to a fine pulp, with frequent dipping of the grated surface in powdered calcium carbonate to neutralize the action of any acids present, and quickly pressed through a tourniquet of several thicknesses of cheese-cloth. The resulting liquid was

diluted with an equal volume of distilled water previously cooled to low temperature, and both solutions packed in ice until ready for use. The only modifications of Appleman's apparatus were the use of test-tubes of about 100 cc. capacity in place of the bottles used by him for gas generation, and the substitution of a small graduated burette of 25 cc. capacity for the separatory funnel serving as the reservoir of hydrogen peroxide.

In determining oxygen values, 1 or 5 cc. of enzyme extract and 5 cc. of fresh solution of commercial hydrogen peroxide (3 per cent H_2O_2) were allowed to react; readings were made every 30 seconds, allowing 15 seconds for gas generation in the chamber and the same time for displacement of the water in the burettes. Before admitting the peroxide solution the enzyme extract previously placed in the gas chamber was brought to 20° C., and this temperature was maintained throughout the series by keeping the chambers in the constant temperature bath. During periods of gas generation and displacement the test-tubes were constantly shaken by hand in as uniform a manner as possible.

Results and Discussion

In the tables and discussion of results the following system of notation is employed for brevity:

Series A 1.—Enzyme dispersion from tissues extracted immediately after etherization,+ substrate.

Series A 2.—Same dispersion, autoclaved before adding to substrate.

Series B 1.—Enzyme dispersion from controls extracted simultaneously with enzyme of A 1,+ substrate.

Series B 2.—Same dispersion, autoclaved before adding to substrate.

Series C 1.—Enzyme dispersion from tissues extracted 18 hours after etherization,+ substrate.

Series C 2.—Same dispersion, autoclaved before adding to substrate.

Series D 1.—Enzyme dispersion from controls extracted simultaneously with enzyme of C 1,+ substrate.

Series D 2.—Same dispersion, autoclaved before adding to substrate.

In table I are given the glucose values obtained by the Shaffer method from the several carbohydrate substrates. The potassium permanganate solution used in the titration was exactly 1/50 normal, 1 cc. being equivalent to 1.272 mg. copper. The amount of sugar per 10 cc. sample of substrate represents the average of two or more titrations,—which did not vary by more than .1–.2 cc. of the permanganate solution,—from which reduction value of the Fehling control has been subtracted.

TABLE I.

THE ACTION OF ENZYMES EXTRACTED FROM ETHERIZED AND UNETHERIZED CORMS OF GLADIOLUS ON CARBOHYDRATES

Substrate		Milligrams sugar as glucose								
		Series number								
		Control	A 1	A 2	B 1	B 2	C 1	C 2	D 1	D 2
Starch	Spl.*	1.2	7.5	1.0	6.6	0.9	2.9	0.7	2.1	0.7
	Tot. sub.†	5.8	36.6	5.2	33.0	4.6	14.6	3.4	10.6	3.4
Dextrin	Spl.	6.0	21.9	5.9	25.6	5.1	13.1	7.9	10.0	14.0
	Tot. sub.	30.0	109.4	29.4	128.2	25.6	65.4	39.4	49.8	70.2
Sucrose	Spl.	22.6 6.4‡	11.4	9.6	8.4	8.9	9.0	10.6	9.7	13.4
	Tot. sub.	113.2 31.8‡	57.0	48.0	42.2	44.4	45.0	52.8	48.6	67.0
Maltose	Spl.	41.2	42.6	42.0	44.3	44.2	42.6	43.6	42.7	42.7
	Tot. sub.	206.2	213.2	210.0	221.6	221.0	212.8	218.0	213.4	213.4
Inulin	Spl.	6.9	6.6	7.4	7.0	6.6	5.8	5.5	5.8	7.8
	Tot. sub.	34.4	33.2	37.0	35.2	32.8	28.8	27.4	28.8	39.2

* Spl.= amount in sample.
† Tot. sub.= total in substrate.
‡ Control minus acid.

The sugar values resulting from the action of the enzyme dispersion on the several carbohydrates are by no means concordant, and in some respects are difficult, if not impossible, to account for adequately. In the case of starch, there appears to be a consistent relation between etherization and an increased hydrolysis. Substrates with enzymes derived from both lots of etherized tissues (A 1 and C 1) are respectively greater than the controls (B 1 and D 1). On *a priori* grounds we should expect no greater starch conversion in the flasks with the previously autoclaved dispersions (A 2, B 2, C 2) than in the control, except such as might be due to hydrolysis by organic acids resulting from the destruction of the dispersion by heat under pressure. We shall see in a consideration of later tables that such increased hydrolysis by the products resulting from disintegration by heat of the enzyme-protein complex appears to be highly probable. In the case of starch, however, the previously autoclaved dispersions seem to have exerted no influence, unless the falling off of the sugar values as compared with the control be regarded as producing an inhibitive or buffer action upon normal processes.

In the case of dextrin the results are not in accord with those just considered, neither are the two halves of the series concordant. The enzyme from the extract made immediately after etherization has been less effective than that from its control; on the other hand, where the corms were allowed to air for 18 hours before extraction, the dispersion from the etherized tissues shows considerable increase in hydrolytic action over its control. The effect of the previously autoclaved dispersions appears to have no great significance here, but attention may be called to the marked increase in the value of D 2 over the other dispersions previously autoclaved. The same relation holds in the series with sucrose and inulin, and seems inexplicable, for if it were due merely to an increased activity resulting from the products of enzyme disintegration, it should have the same relative value with respect to C 2 that B 2 has to A 2,—which is not the case. We are not warranted in explaining the differences in the two halves of this series,—immediate extraction *versus* extraction after air-

ing,—on the hypothesis that such airing has caused an increased conversion of zymogen to enzyme in the case of the etherized corms, because the data for the other carbohydrates show values directly opposed to dextrin in this respect. In view of the relation of dextrin to starch as an intermediate product of the hydrolysis of the latter, the results of the dextrin series are extremely puzzling.

In a consideration of the figures for sucrose it should again be noted that a small amount of acid was added to all substrates in order to make conditions for invertase action more favorable. With this in view, an additional control without acid was used. The latter indicates that in the absence of inhibiting factors, over 71 per cent of the resulting inversion was due to the acid added. That there were inhibiting factors of some nature appears from a study of the remaining figures in the sucrose columns. The relation between the dispersions from etherized and unetherized tissue is apparent, although here also, as in dextrin, there is a contradiction between the two halves of the series. The only plausible explanation lies in the assumption either that no invertase was present in the corms, and that the organic constituents of the protein-enzyme complex merely acted as a buffer on the hydrolytic activity of the hydrochloric acid added to the substrate, or that the invertase present in the corms was either not extracted or was inactivated by the methods employed. The latter assumption appears even less warranted than the first, since the experience of many students of invertase shows that it is one of the enzymes most readily extracted by water.

With respect to the action of the dispersion upon the maltose substrate it appears reasonably clear that there has been no maltase activity, and it is possible that the enzyme was not present. In this case, however, there is experimental evidence that lends support to the belief that the maltase present may have been destroyed in the process of extraction, since Daish ('16) found that the maltase present in air-dried germinated barley was destroyed by extraction with water and subsequent precipitation with alcohol. The figures for inulin show the same general relations of enzyme from ether-

ized tissues and controls that have been noted in the case of dextrin.

The conclusion seems unavoidable, in the light of the results above noted, that for a study of the effect of etherization upon carbohydrates a method must be employed that obviates the various undesirable factors involved in the processes of enzyme extraction. Such extraction and the filtration of dense colloidal plant solutions involve long periods of time which are undoubtedly unfavorable to enzyme isolation. There is the added objection, also apparent in the foregoing table, that there is no means of concluding, in the absence of positive results, whether the enzyme is not present in the tissues or is not amenable to extraction by the method used. Additional data on carbohydrate enzymes with respect to their action following etherization have been obtained in a supplementary series of experiments in which the foregoing difficulties have been eliminated; the results of this series will be discussed later.

The titration of 10-cc. portions of the substrates of ethyl acetate, ethyl butyrate, and oil emulsion, with sodium hydrate showed total absence of lipolytic activity. This was not unexpected, for several reasons. In the first place, experiments showing lipase action have in the past been made for the most part with material of high fat or oil content, such as seeds, especially those of *Ricinus,* rather than with storage organs in which the carbohydrates predominate, such as corms, bulbs, and tubers. Experience has shown that in general, under the most favorable conditions, the amount of active enzyme preparation extracted from plant tissues is quite small in proportion to the amount of material used. The most notable exception in this respect is doubtless the urease derived from several members of the bean family. The chances of obtaining an active lipase preparation from organs such as those used in the present study, even with the most favorable preparation methods, were therefore small.

In the second place, most lipases, as pointed out by Euler ('12), are insoluble in water. Berczeller ('11) concluded that lipase of the pancreas does not go into solution and that the

enzyme acts in a suspension. Oppenheimer ('13) goes further and states that lipases are insoluble in water, glycerin, fats, or ethereal solvents; that the enzyme always acts in suspension. To insure lipolytic action it is therefore desirable either to bring the desiccated plant material containing the enzyme,—preferably after extraction of fats,—directly in contact with the substrate, as suggested by the work of Connstein, Hoyer and Wartenberg ('03) and by Wohlgemuth ('13), or to follow the method cited by Haas and Hill ('17) for extracting the enzyme for commercial usage. In the latter process the seeds or material of high oil content are ground with water, centrifuged, and the resulting emulsion of oil, protein, and enzyme allowed to ferment at a moderate temperature until a scum containing the enzyme rises to the surface. Hydrolysis is effected by allowing this scum to act upon fats in the presence of water and manganese sulphate as a catalyser. In the present study it was not possible to follow either of the methods outlined above for lack of sufficient corms of the kind used in the experiment, and it was necessary to use in the series the enzyme dispersion in aqueous extract to observe a possible, rather than a probable, reaction.

In the following table showing the relations of enzyme extract to acetamid and asparagin the values presented are

TABLE II.

THE ACTION OF ENZYMES EXTRACTED FROM ETHERIZED AND UNETHERIZED CORMS OF GLADIOLUS ON AMIDO AND AMINO COMPOUNDS

Series number	Milligrams ammonia nitrogen from			
	Acetamid		Asparagin	
	In sample	In total substrate	In sample	In total substrate
Control	.0474	2.370	.0396	1.980
A 1	1.7640	88.200	.0388	1.940
A 2	.0476	2.380	.0387	1.935
B 1	.1417	7.085	.0360	1.800
B 2	.0377	1.885	.0248	1.240
C 1	.0423	2.115	.0395	1.975
C 2	.0452	2.260	.0371	1.885
D 1	.0376	1.880	.0313	1.565
D 2	.0406	2.030	.0249	1.245

based upon the colorimetric determinations of 1 cc. of the Nesslerized substrate, measured from an Ostwald precision pipette. The data are the averages of at least two, and in some cases three, such determinations. Two flasks were Nesslerized for each flask of substrate, and four colorimeter readings made from each Nesslerized solution, the ammonia nitrogen being then computed from the average reading. In the case of the acetamid substrate the Nesslerization of the series was repeated to insure a check on the results obtained in the first run.

The results here point to a very definite and a surprisingly great effect of etherization upon the subsequent activity of amidase. The amount of ammonia nitrogen split from acetamid by the enzyme from the etherized tissues is more than twelve times that obtained from the corresponding control. The values for the autoclave dispersions are consistent and correspond closely with that of the control. In the half of the series in which the corms were allowed to air for 18 hours before extraction, there appears no increased action over the control. This tends toward the conclusion that in the time following etherization the period of stimulus resulting from the anaesthetic has been followed by a return to normal conditions, or that during such time the enzyme has completed its activity before extraction.

The data for asparagin, on the other hand, show a practical absence of any enzyme action. The values are in all cases small, and a very accurate distinction between the several Nesslerized solutions was difficult because of the slight amount of color present. It appears clear, however, that there is a marked specificity of action on the part of the enzymes splitting off ammonia.

The analyses of the protein substrates in neutral and alkaline solution for the action of proteolytic enzymes follow. The figures were obtained by translating the volume of nitrogen gas evolved at the observed temperature and pressure into terms of amino-nitrogen in milligrams, using for this purpose the tables of Gattermann ('10) and dividing the values there given by two. Two flasks were unfortunately lost during the

time of incubation by the blowing out of the corks and consequent evaporation.

TABLE III

THE ACTION OF ENZYMES EXTRACTED FROM ETHERIZED AND UNETHERIZED CORMS OF GLADIOLUS ON PROTEINS

Series number	Milligrams amino-nitrogen from albumin			
	Neutral		Alkaline	
	In sample	In total substrate	In sample	In total substrate
Control.....	.2918	7.294	.2923	7.307
A 1.....	.4292	10.730	.8291	20.727
A 2.....	.2951	7.377	.3933	9.837
B 1.....	.2307	5.767	.4285	10.712
B 2.....	.2200	5.500	.2486	6.215
C 1.....	.3970	9.925	†	.....
C 2.....	.2414	6.035	.2232	5.580
D 1.....	.2993	7.482	.2551	6.378
D 2.....	.2762	6.680	.3455	8.637

Series number	Milligrams amino-nitrogen from							
	Casein				Peptone			
	Neutral		Alkaline		Neutral		Alkaline	
	In sample	In total substrate	In sample	In total substrate	In sample	In total substrate	In sample	In total substrate
Control...	.6449	16.122	.4642	11.605	.6120	15.300	.6396	15.990
A 1.....	.9797	24.492	.7427	18.567	.8605	21.512	.8064	20.165
A 2.....	.7249	18.122	.6790	16.975	.5810	14.525	.5570	13.975
B 1.....	.8848	22.120	1.4058	35.145	.9381	23.452	.8545	21.362
B 2.....	.9541	23.852	.4774	11.935	.8117	20.292	.8018	20.045
C 1.....	.7904	19.760	.6631	16.577	.6737	16.842	.9178	22.945
C 2.....	1.2060*	30.150*	.4297	10.742	.7056	17.640	1.0022	25.055
D 1.....	.7427	18.567	†	.....	.7109	17.772	.9534	23.835
D 2.....	.8929	22.322	.5835	14.587	.6544	16.360	.7016	17.540

* Autoclaved twice after incubation.
† Lost by evaporation in incubator.

The results here vary considerably with the substrate and with the reaction of the solution. In the case of albumin a definite increase in amino-nitrogen may be noted in the flasks acted upon by enzymes from etherized corms, in both halves of the series and in alkaline as well as in neutral media. In the former the action has been practically twice as great as in the neutral albumin. In casein the amino-nitrogen values are as a whole considerably higher than for albumin, but a comparison of the respective controls shows that this may be

largely accounted for by the greater normal hydrolysis of the casein. In neutral solution the dispersion from etherized tissues again shows a greater activity than that from the unetherized corms. In alkaline solution A 1 is markedly less than B 1. Whether this is due to the condition of the medium with respect to the enzyme cannot be determined, unfortunately, since the flask of enzyme control of the second half of this series, D 1, was lost during incubation. It is conceivable that such a great difference might be the result of an error in adding the enzyme dispersion twice to the same flask in preparing the series, although care was taken in this respect. In the peptone substrates, both alkaline and neutral, a retardation or inhibition of the activity of the enzyme from etherized tissues may be noted. This holds for both halves of the series. As a whole, the amount of hydrolysis in the peptone series bears a close relation to that obtained with casein, and is again much greater than that of albumin. In view of the consistent relation of the results with albumin and peptone, in the two halves of a series as well as in acid and alkaline media, we are inclined to ascribe the increased value of B 1 over A 1 in the casein alkaline series to an error of manipulation rather than to any effect upon enzyme action.

In a consideration of the carbohydrate experiments attention was called to the probable effect on hydrolysis of the previously autoclaved dispersions. This appears in several instances in the table above, but the effect is by no means uniform. In three cases (albumin alkaline D 2, and casein neutral B 2 and D 2) it relates to the dispersion from unetherized tissues, and in three others (casein neutral C 2, and peptone neutral C 2 and alkaline C 2), to the controls. No explanation seems sufficient to account for an increase in value obtained from these flasks over those with the corresponding letters, unless such increase is due to the products resulting from the disintegration of the previously autoclaved dispersion. In support of this probability is the value for casein neutral C 2, which, after the second autoclaving, was by mistake subjected to a third autoclaving by another worker in the laboratory. It would appear that the increased number

of subjections to high temperature bears a more or less direct relation to the hydrolysis effected.

The ultimate solution of the coagulated albumin in alkaline media was to be expected, but it had been hoped to obtain comparative data regarding the effect of the enzyme dispersions before complete solution resulted,—and complete solution occurred before the tenth day after incubation began. Observations at intervals of several days, however, showed no detectable difference in the amount of albumin dissolved by the alkaline water of the control and in the flasks containing enzyme dispersion. The results in the case of tubes in acidified water are given below. The average values given in the last column were obtained from the total digestion in three tubes in each flask.

TABLE IV

THE ACTION OF ENZYMES EXTRACTED FROM ETHERIZED AND UNETHERIZED CORMS OF GLADIOLUS ON COAGULATED ALBUMIN IN DIGESTION TUBES IN ACIDIFIED WATER

Series number	Total length in mm. of albumin cylinders digested	Average length in mm. digested from 6 ends of cylinders 2 mm. in diam.
Control	Not measurable	.....
A 1	2.0	0.333
A 2	Not measurable	.....
B 1	12.5	2.083
B 2	Not measurable	.....
C 1	0.5	0.083
C 2	Not measurable	.....
D 1	5.5	0.916
D 2	Not measurable	.....

The relative reactions of the dispersions are here exactly the reverse of those occurring in neutral or alkaline solutions of albumin. While there has been some action on the part of all enzyme mixtures not previously autoclaved, the activity of those from the two lots of unetherized tissues has been notably greater than of those from etherized corms. The results are not readily explained. If the acidity of the medium were in itself the inhibiting factor, an inhibition of both dispersions might be looked for. It would seem, therefore, as if the mixture of enzyme and protein extracted from the etherized tissues differed chemically from that obtained from the control

corms, and that because of such difference the action of the former was retarded in the presence of the acid.

Table v is concerned with the results of the catalase experiments with fresh extracts from the etherized and the control corms.

TABLE V

CATALASE ACTION OF FRESH EXTRACTS OF ETHERIZED AND UNETHERIZED GLADIOLUS CORMS ON HYDROGEN PEROXIDE

Concentration	Time intervals in minutes	Cubic centimeters oxygen from	
		Catalase from etherized corms	Controls
I. 1 cc. extract 1 cc. water 5 cc. H_2O_2	0.5	1.7	2.7
	1.0	1.2	1.3
	1.5	0.8	1.1
	2.0	0.9	0.8
	2.5	1.0	0.8
	3.0	0.5	0.7
Total...	3.0	6.1	7.4
II. 5 cc. extract 5 cc. H_2O_2	0.5	2.0	11.1
	1.0	3.5	12.2
	1.5	3.3	3.3
	2.0	2.5	2.9
	2.5	2.1	2.5
	3.0	1.8	2.1
	3.5	1.7	2.0
	4.0	1.4	1.6
	4.5	1.3	1.5
Total...	4.5	19.6	39.2
III. 5 cc. extract 5 cc. H_2O_2	0.5	16.6	12.0
	1.0	3.2	8.2
	1.5	2.6	4.9
	2.0	2.5	2.8
	2.5	2.1	1.2
	3.0	1.2	1.8
	3.5	1.5	1.6
	4.0	1.0	1.4
	4.5	1.1	1.1
	5.0	1.1	1.1
Total...	5.0	32.9	36.1

The results of the catalase determinations point conclusively to an inhibition of catalytic activity immediately following the period of etherization. This accords in part with the findings of Burge ('17) in his studies of blood catalase. Whether the enzyme returns to normal activity if the corms

are permitted to air for some time after etherization could not be determined with the material at hand, but this is a point worthy of study. In regard to the results obtained, it is of interest to note that they are related to the conclusions of Palladin ('10) and Appleman ('10, '15). The former concluded from experiments with *Vicia Faba* that toxic agents acted as inhibitors of respiration; the latter investigator found that catalase action in the potato bore a direct relation to respiratory activity,—decreasing under the same conditions as respiration.

EXPERIMENTS WITH BARLEY

In view of the inconclusive and inconcordant results obtained from the action of the *Gladiolus* extracts upon the several carbohydrate substrates, it seemed advisable to carry out a supplementary series of experiments with germinated barley, using methods which should exclude the introduction of indeterminable factors of error, or which would at least indicate the source and amount of the error resulting from such methods. An experimental test of the Dubois ('83) theory of the dehydration of tissues by anaesthetics was also made at this time.

The material used was seed of barley (*Hordeum vulgare*). Only plump and apparently viable grains were selected. These were disinfected by the calcium hypochlorite method of Wilson ('15), as modified by Dr. Duggar in this laboratory. The seeds were treated for one hour with a 20 per cent solution of Javelle water, rinsed in tap water, and germinated between moist filter paper. With this treatment germination of more than 95 per cent was secured. A preliminary trial of seeds in 0.1 per cent aqueous solution of mercuric bichloride for two hours proved to be highly injurious, not more than a two or three per cent germination resulting. This is a very much weaker solution than that used by Brown ('09) in his study of seed-coat permeability.

In all cases the seeds were allowed to germinate until the plumules had reached a length of 1–1.5 cm. and the rootlets 1 cm. or less. For the dehydration test two lots of germinated

seeds, 125 in each lot, were used, one being etherized, the other serving as control. The procedure and dosage were the same as in the foregoing experiments, except that the jars containing the material stood at room temperature of about 30° C. Before placing in the jars each lot of seed was wiped between filter paper to remove excess moisture, and weighed. Both jars contained a filter paper sufficiently moist to prevent injury to the seeds by drying out. At the close of the experiment the seeds were again wiped and weighed. The following data were obtained:

TABLE VI

EFFECT OF ETHERIZATION ON THE WEIGHT OF GERMINATING BARLEY

Treatment	No. of seeds	Original weight in gms.	Weight in gms. at end of experiment	Gain in weight		Remarks
				Gms.	Per cent	
Etherized	125	8.43	9.95	1.52	18.0	Plumules 1.5–2 cm. long, etiolated. No growth of radicles.
Control	125	7.90	9.50	1.60	20.2	Plumules 2–3.5 cm. long, partly green. Radicles about same length.

It is evident from the figures above that there was no dehydration as the result of etherization. On the contrary, both lots of seed took up water from the paper in the jars, as indicated by the definite gains in weight. The relative difference in gain, however, is not especially significant, and probably falls within the range of experimental error, considering the fact that in both weighings moist seeds were involved. The most definite results appear in the difference in growth of the two lots of seeds, as indicated in the last column of the table. We have here a corroboration of the findings of other workers in this field, of the inhibitive effect of ether on germinating seeds.

For the experiments with carbohydrate substrates, two lots of selected barley seeds, weighing 150 grams each, were dis-

infected and germinated as already noted. The jars for both lots contained sufficiently moist filter paper to supply the necessary amount of water for the seeds during the usual 24-hour period of etherization, and during this time stood at room temperature of 28–32° C. At the end of the period both the etherized and control seeds were quickly dehydrated by immersion in 50 per cent alcohol for 5 minutes, for 10 minutes in each of two changes of 95 per cent alcohol, and then for 10-minute periods in acetone, 95 per cent alcohol and acetone in the order named. They were then air-dried before an electric fan at high summer temperature. When thoroughly dry both lots were twice ground into coarse meal in a food grinder and finally made into a fine flour by grinding twice in a mill, after which they were stored in desiccators.

The substrates used were a 1 per cent starch solution, made as outlined in the previous work, sucrose (Merck), and maltose (Merck), each in 2 per cent solution. Fifty cc. of each substrate with 1 per cent toluol were used in each flask, and to them were added 5 grams of the respective lots of barley flour. The tests were run at room temperature of 30° C. A preliminary test for amylases was made to determine the length of time desirable to run the series. By the use of the iodine spot-plate test for starch it was found that a little more than $3\frac{1}{2}$ hours elapsed before the action of 5 gms. of either flour acting on 50 cc. of starch solution failed to give positive results. The flasks were therefore allowed to stand for $1\frac{3}{4}$ hours after adding the flour in the case of the starch, and for 2 hours in the case of sucrose and maltose.

Two flasks of each substrate with flour from etherized barley and a like number with control flour were employed. Fifty cc. of each solution served as a check upon the substrate itself. At the end of the run one flask with etherized and one with control flour were at once heated to 15 pounds pressure in the autoclave, cooled, the mixture centrifuged, and the liquid analyzed for reducing sugars by the Shaffer method. The other pair of flasks was similarly treated, except that the contents were first centrifuged, and the liquid alone then autoclaved. It was believed that a comparison of the values from

the respective flasks would perhaps throw some light upon the effect that autoclaving might exert upon the organic matter present in the flours, as indicated in the sugar determinations.

In the table below the following notation is used:

C.—Substrate with flour from unetherized barley.

Cf.—Substrate with flour from unetherized barley, flour removed before autoclaving.

E.—Substrate with flour from etherized barley.

Ef.—Substrate with flour from etherized barley, flour removed before autoclaving.

Sb.—Substrate control.

TABLE VII

THE ACTION OF FLOUR MADE OF ETHERIZED AND UNETHERIZED GERMINATING SEEDS OF HORDEUM ON CARBOHYDRATES

Substrate		Milligrams sugar as glucose				
		Series letter				
		C	Cf	E	Ef	Sb
Starch	Spl.*	23.53	22.19	22.80	21.49	0.38
	Tot. sub.†	117.65	110.95	114.00	107.45	1.90
Sucrose	Spl.	21.12	21.83	24.76	20.09	8.18
	Tot. sub.	105.60	109.15	123.80	100.45	40.90
Maltose	Spl.	38.63	39.53	37.33	37.82	26.53
	Tot. sub.	193.15	197.65	186.65	189.10	132.65

* Spl.= amount in sample.
† Tot. sub.= total in substrate.

The sugar data above show a consistently lower value for all substrates with flour from etherized seeds, where the substrate was autoclaved after being separated from the solid constituents of the barley. This is also true in most of the substrates where the flour and the solutions were heated together, but there is a marked deviation in the case of sucrose, where E is considerably greater than C. On the other hand, Cf, conforming to the relations holding in the other substrates, is larger than Ef. It is also evident that the relations of C

to Cf and of E to Ef are not the same in the different substrates. In starch, C exceeds Cf, but is less than Cf in sucrose and maltose. E is greater than Ef in starch and sucrose, but falls below Ef in maltose. There seems to be ground for the conclusion that the heating of a substrate containing the enzyme dispersion or the enzyme powder results in the introduction of a disturbing factor that gives inconcordant titration values. On the other hand, the uniform relations between the respective figures for the substrates where the clear liquid was autoclaved lend support to the belief that the removal of the material containing the enzyme before the substrate is heated tends to eliminate or reduce such a factor of error.

Considering the Cf and Ef data alone, therefore, it would appear that in the case of barley the effect of ether upon the germinating seeds is an inhibition of some of their hydrolytic activity, as expressed in terms of action upon an external substrate. This difference in enzyme activity conforms to the differences in growth noted in table v, where control seeds show greater growth than etherized ones. The results support the findings of Lintner and Kröber ('95) and of Eisenberg ('07).

CONCLUSIONS

The experiments here reported may be regarded as a preliminary study of the many questions involved in the problem of anaesthetic action on enzyme activities in plants. The results obtained, although difficult to interpret satisfactorily in the case of some substrates,—notably the carbohydrates in the experiments with *Gladiolus*,—have pointed the way to improved methods of attack. Aside from the substrates just noted, definite results have been obtained with enzyme dispersions acting upon protein and amido substances, and with catalase. The general conclusions appear warranted that tryptic or ereptic enzymes and an amidase were extracted, and that the activity of the dispersions containing these enzymes was in some instances stimulated as the result of etherization; in other cases, as with catalase and with the carbohydrases of germinating barley, the anaesthetic seems to have effected inhibitory action. A very marked increase in action on aceta-

mid following etherization appears concordant with the findings of Willaman ('17) with respect to the effect of ether on enzymes producing cyanogenesis. The experiments do not support the opinion of Johannsen ('97) and do tend to confirm the view of Green ('87) in regard to the conversion of zymogen to enzyme as the result of anaesthetic stimulation. In the case of germinating barley, etherization has resulted either in reduction of the rate of zymogen conversion, or possibly in the production of substances having the properties of anti-enzymes. No conclusions can properly be deduced from a comparison of the results obtained with *Gladiolus* and *Hordeum*. They must be considered separately, for a factor that cannot be neglected is the relation of enzyme response to the rest period of the plant. The assumption is warranted that at certain stages in the life cycle of plants with regular rest periods there will be times of normal maximum and minimum enzyme activity, and the results obtained in etherization experiments will depend upon the application of the narcotic at a time properly related to such normal enzyme conditions. The use of organs with practically indefinite or indeterminate rest periods, such as grain seeds, introduces altogether different conditions. In connection with this phase of the question a study of the effects upon enzyme action resulting from etherization at different periods in the annual cycle of some plant would prove of much value.

Summary

An historical review of the literature of experiments dealing with the responses of plants to anaesthetics is presented, and the several theories of narcosis and of the relation of narcotics to enzyme activity are reviewed.

The methods of experimentation are described.

Among the experimental results obtained, the following are the most definite:

Enzyme dispersions from etherized corms of *Gladiolus* were distinctly more active upon acetamid than dispersions from controls, the ratio of ammonia nitrogen split off from the two series being about 12 to 1. No difference was noted in the

action on asparagin; a specificity of the extracted enzyme is thus indicated.

Dispersions from etherized tissues showed increased proteolytic action on albumin in neutral and in alkaline solutions, and on casein in neutral solutions, as compared with enzymes from controls. With peptone the reverse action was noted, both in neutral and in alkaline media. Action upon coagulated albumin in acid medium was greater in controls than in the dispersions from etherized corms.

Catalase activity appeared to be inhibited as the result of etherization.

Of the experiments with enzyme dispersions from *Gladiolus* on carbohydrates, starch was the only substrate showing consistent results, and these pointed to increased hydrolysis following etherization. Data from the other carbohydrates are conflicting and do not warrant definite conclusions.

The action on carbohydrates of barley flour from etherized and unetherized germinated seeds showed a uniform inhibition resulting from the anaesthetic. It was also shown that anaesthetics do not effect dehydration.

The various data in this study warrant the conclusion that the presence of the colloidal enzyme-protein complex or of solid organic matter, such as meal or flour from tissues, introduces an undesirable source of error if, at the time that enzyme action must be stopped, these substances are heated together with the substrate. The experiments with barley indicate that inhibiting enzyme action after freeing the substrate from insoluble organic matter tends to give more concordant results.

The results as a whole tend to confirm the view that anaesthetics exert a more or less direct influence upon the subsequent activities of plant enzymes.

Acknowledgments

The writer takes pleasure in acknowledging his indebtedness to all who have contributed their support to the prosecution of the present studies. Sincere thanks are extended to Dr. George T. Moore for the privileges of the laboratory and

library of the Missouri Botanical Garden; to Dr. B. M. Duggar, under whose direction the work was carried out, for interest and kindly criticism and advice; to the Department of Biological Chemistry and the Medical School of Washington University for laboratory facilities, and to Dr. S. M. Zeller, Visiting Fellow in the Henry Shaw School of Botany, for helpful assistance in the catalase determinations. Acknowledgment is also due Mr. J. Christian Bay, of the John Crerar Library, Chicago, for a careful translation and abstract of the Danish paper by Johannsen.

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